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 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 phase HPLC method, ICH guidelines, Method validation.

Introduction

Mycobacterium tuberculosis (TB) is a well-known bacterium that causes tuberculosis and is also known as a superbug [1]. Tuberculosis (TB) is a disease that affects not just the lungs but also other organs in the body. Tuberculosis is believed to infect one-third of the world's population, with a yearly prevalence of 1% of the total population [2]. Out of a total of 10 million active TB infections, 1.3 million fatalities were reported in 2016. Over 95 percent of deaths occurred in developing countries, with South Asian countries such as India, China, Pakistan, Indonesia, and the Philippines accounting for 50 percent [3, 4]. In 2012, the USFDA authorised bedaquiline (BDQ), a quinolone derivative, as a new chemical for the treatment of Mycobacterium TB. By 2015, BDQ has been adopted by all developing countries, and it is now being used to treat multidrug-resistant tuberculosis (MDRTB) alone

or in combination with other antibiotics [5-7]. It is chemically [(1R, 2S) 4-(dimethylamino)-2-(1-naphthalenyl)-1-phenyl-2-butanol -1-(6-bromo-2 methoxy-3-quinolinyl)-4-(dimethylamino)-2-(1-naphthalenyl)-1-phenyl-2-butanol (Fig. 1). BDQ kills the superbug by inhibiting the ATP synthase enzyme, which is required for energy production. The cytochrome P450 enzyme CYP3A4 is in charge of BDQ metabolism, which results in M2 as the primary metabolite with a 6 fold decrease in activity [8]. BDQ tablets are now available in 100 mg dosages, which must be taken four times per day for the first two weeks of therapy, followed by 200 mg doses three times per week with a minimum 48-hour interval between doses for the duration of treatment. The medication should be provided under strict monitoring, with the special recommendation to save BDQ for cases in which an effective TB regimen is not available [9, 10]. BDQ is classified as having low water solubility by the Biopharmaceutical Classification System (BCS). Its bioavailability/dissolution rate is impeded (limited) when taken orally [11]. The FDA specifies 0.01 N HCl as the official dissolving medium for BDQ, whereas methanol is typically used as a solvent to form the stock solution during analytical technique development. As a result, during the length of the inquiry, BDQ must be stable in official dissolving medium and methanol. Spectrophotometric approaches [12, 13], forced degradation research [14, 15], simultaneous estimates [16, 17], bio-analytical HPLC/MS methods [16, 17], chiral analysis [18, 19], pharmacokinetic studies [20-22], and simultaneous estimates. This work covers the invention and validation of a 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.

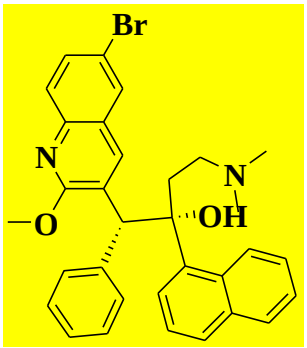


Figure 1 Chemical structure of Bedaquiline

MATERIALS AND METHODS

Instrumentation

Waters 784 liquid chromatographic system with manual injector, Waters 515 binary pump for constant flow and constant pressure supply, and UV-Visible detector linked to Data Ace

software for instrument management and data processing. The Citizen Scale (I) Pvt. Ltd. Digital Micro Balance was used for the weighing (CX-265).

Chemicals and reagents

Dishman Pharmaceuticals and API, both based in Ahmedabad, Gujarat, India, graciously contributed an analytically pure sample of BDQ as well as their analytical results. Methanol and acetonitrile were supplied by Rankem, RFCL Limited, New Delhi, India. Ammonium acetate AR, sodium dihydrogen phosphate AR, and ortho-phosphoric acid AR grade were supplied by Central Drug House (P) Limited, New Delhi, India. The 0.45m pump nylon filter was supplied by Advanced Micro Devices (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), running through the column at a constant flow rate of 1.0 ml/min. The mobile phase was degassed and filtered using nylon 0.22m membrane filters prior to use (30 min). A Thermo (C-18) column used as the stationary phase (5 m, 250mm x 4.60mm). The UV-Visible detector's detection wavelength was determined based on the chromatographic parameter, sensitivity, and selectivity of the technology for pharmaceuticals.

Selection of Mobile Phase

Initially, several mobile phase ratios were utilised to calculate bedaquiline in bulk medicine. Taking into account system appropriateness characteristics such as RT, Tailing factor, No. of theoretical plates, and HETP, the mobile phase determined to be most suitable for analysis was 10mM Ammonium acetate buffer: methanol in the ratio of 15:85v/v (pH adjust with 4.5 with OPA). The mobile phase was filtered through 0.45mm filter paper and then degassed using sonication to remove particle material. A flow rate of 1.0 ml/min was employed for the analysis.

Diluent Selection

The diluent used to prepare the sample was compatible with the mobile phase, and it had no influence on the retention or resolution of the analyte. Following multiple attempts, 6.8 pH Phosphate Buffer was used as a diluent.

Preparation of Stock Solution

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

was brought up to 10ml with phosphate buffer pH 6.8 to get a solution concentration of 1000mg/ml (Stock-A).

Sub Stock Solution Preparation

5 ml of each drug's stock-A solution was put into a 50ml volumetric flask separately and diluted up to 50 ml with diluent (phosphate buffer pH 6.8) to yield a concentration of 100g/ml of BDQ (Stock-B).

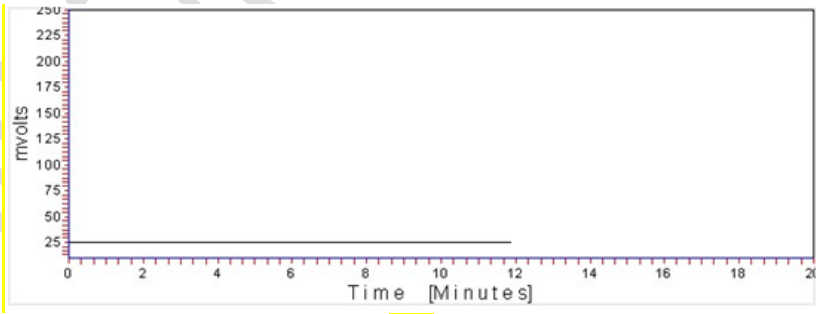
Preparation of Different Solution

0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml of stock-B were taken separately in 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.

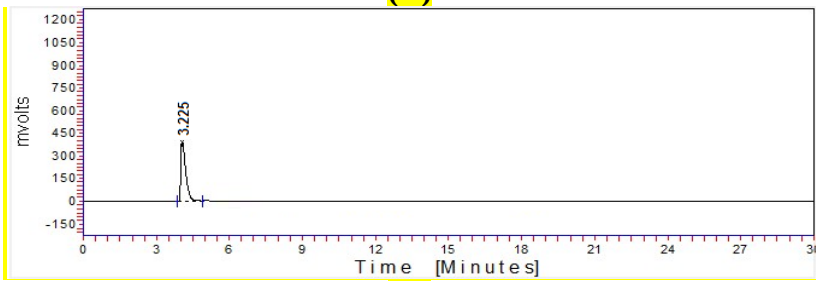
Results and discussion

Chromatography

The mobile phase was chosen after many attempts with various proportions and pH values of methanol, isopropyl alcohol, acetonitrile, water, and buffer solutions. A mobile phase of 10mM Ammonium acetate buffer: methanol in the ratio of 15:85v/v (pH adjusted to 4.5 with OPA) was used to achieve maximal separation and sensitivity. Flow rates ranging from 0.5 to 1.5 minutes were studied. The signal-to-noise ratio was outstanding and the separation time was adequate with a flow rate of 1 ml/min. The retention periods for BDQ were found to be 3.225 0.002min using a reversed-phase C18 column. The entire analysis took less than 5 minutes. The greatest absorption of BDQ was measured at 232nm, and this wavelength was used in the study (Figure 2).



(A)



(B)

Fig. 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 preanalysed 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 ICH guidelines to test the method's ability to stay unaffected. The ratio of mobile phase was change from, 10mM Ammonium acetate: methanol (15:85% v/v) to (20:80 % v/v) and 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 level)

Detection Limit and Quantitation Limit

The LOD and LOQ of 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 suggested HPLC technique was verified in accordance with the ICH Q2B Guidelines and was determined to be appropriate for routine quantitative measurement of BDQ in pharmaceutical dosage form by HPLC. All of the linearity, precision, accuracy, and specificity values were determined to be satisfactory. The method enables selective BDQ quantification. The suggested approach was very repeatable, trustworthy, quick, robust, and exact. As a consequence, with a high percentage of recovery and a run time of less than five minutes, it is suitable for routine BDQ detection 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 use 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 personal efforts of the authors.

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