Analytical Method Development and Validation for Estimation of Bedaquiline (BDQ) Using RP-HPLC

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Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 3.225 minutes 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.

1. 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 only 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 \((1R, 2S) 4-(\text{dimethylamino})-2-(1-naphthalenyl)-1-phenyl-2-butanol -1-(6-bromo-2-methoxy-3-quinoliny)-4-(\text{dimethylamino})-2-(1-naphthalenyl)-1-phenyl-2-butanol\) (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.

2. MATERIALS AND METHODS

2.1 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).

2.2 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.
2.3 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.

2.4 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.

2.5 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.

2.6 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).

2.7 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).

2.8 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.

3. RESULTS AND DISCUSSION

3.1 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 (Fig. 2).
3.2 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

3.3 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:

\[ Y \text{ (BDQ)} = 50.02 \text{conc} + 11.52 \quad (r^2 = 0.999) \]

3.4 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

3.5 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.

3.6 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 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 (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)

3.7 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        |

4. 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.

DISCLAIMER

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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nazir T, Abraham S, Islam A. Emergence of potential superbug Mycobacterium tuberculosis, lessons from New Delhi mutant-1 bacterial strains. Int J Health Sci (Qassim). 2012;6(1):87–94.
2. Lee SH. Tuberculosis infection and latent tuberculosis. Tuberc Respir Dis (Seoul). 2016;79(4):201–206.
3. Jones JG, King TC. Latent mycobacterium tuberculosis infection. N Engl J Med. 2015;373(12):1178.
4. Raviglione MC, Kochi A, Snider DE. Global epidemiology of tuberculosis: morbidity and mortality of a worldwide epidemic. JAMA. 1995;273(3):220–226.
5. Janssen Therapeutics. Sirturo™. Available:http://www.sirturo.com/
6. Annabel K. GHE, UK. Available:https://tbfacts.org/bedaquiline/2020; (Accessed February 20, 2019).
7. World Health Organization (WHO). Tuberculosis; 2019. Available:https://www.who.int/tb/challenges/mdr/bedaquiline/en/ (Accessed March 14, 2020).
8. Yadav S, Rawal G, Baxi M. Bedaquiline: A novel antitubercular agent for the treatment of multidrug-resistant tuberculosis. J Clin Diagn Res. 2016;10(8):FM01–FM02.
9. Svensson EM, Aweeka F, Park JG, Marzan F, Dooley KE, Karlsson MO. Model-based analysis of the effects of efavirenz on bedaquiline pharmacokinetics and suggested dose adjustments for patients coinfected with HIV and tuberculosis. Antimicrobial Agents and Chemotherapy. 2013;57(6):2780–2787.

10. Fiorillo M, Lamb R, Tanowitz HB, Cappello AR, Martinez-Outschoorn UE, Sotgia F, Lisanti MP. Bedaquiline, an FDA-approved antibiotic, inhibits mitochondrial function and potently blocks the proliferative expansion of stem-like cancer cells (CSCs). Aging (Albany NY). Aging (Albany NY). 2016;8(8):1593-607.

11. Xia X, Pethe K, Kim R, Ballell L, Barros D, Cechetto J, HeeKyoung Jeon, Kideok Kim, Alfonso E, Garcia-Bennett. Encapsulation of antituberculosis drugs within mesoporous silica and intracellular antibacterial activities. Nanomaterials. Nanomaterials (Basel). 2014;4(3):813–826.

12. Pooja BS, Shetty SS. Development and validation of UV spectrophotometric methods for the estimation of bedaquiline in bulk and pharmaceutical formulations. World Journal of Pharmaceutical Research. 2018;7(7):1579–1586.

13. Pardhi V, Pant G, Flora SJS. RP-HPLC method development and validation of bedaquiline fumarate to evaluate its forced degradation behavior and stability in official dissolution media; Future Journal of Pharmaceutical Sciences. 2020;6(42):1245–1251.

14. Momin MA, Rangnekar B, Das SC. Development and validation of a RP-HPLC method for simultaneous quantification of bedaquiline (TMC 207), moxifloxacin and pyrazinamide in a pharmaceutical powder formulation for inhalation; Journal of Liquid Chromatography and Related Technologies. 2018;41(8):415–421.

15. Gray WA, Waldorf B, Rao MG, Stiles BL, Griffiss JML, Salata RA, et al. Development and validation of an LC-MS/MS method for the simultaneous determination of Bedaquiline and Rifabutin in human plasma. Journal of Pharmaceutical and Biomedical Analysis. 2019;176:112775.

16. Alffenaar JWC, Bolhuis M, Van Hateren K, Sturkenboom M, Akkerman O, De Lange W, et al. Determination of bedaquiline in human serum using liquid chromatography–tandem mass spectrometry; AAC. 2018;59(9):5675–5680.

17. Hui Z, Zhong-quan L, Li X, Shao-chen G, Bin W, Lei F, et al. Determination of Bedaquiline plasma concentration by high performance liquid chromatography – mass spectrometry/mass spectrometry; Chinese Journal of Antituberculosis. 2018;40(12):1319.

18. Dousa M, Reitmajaer J, Lustig P, Stefko M. Effect of chromatographic conditions on Enantio-separation of Bedaquiline using polysaccharidebased chiral stationary phases in RP-HPLC. Journal of Chromatographic Science. 2016;54(9):1501–1507.

19. Jiang S, Wu C, Yu J, Sun T, Guo X. Separation of eight bedaquiline analogue diastereomers by HPLC on an immobilized polysaccharide-based chiral stationary phase; Chirality. 2019:31(1):72–78.

20. Gone V, Thakur S, Valupadasu N, et al. Pharmacokinetic study of bedaquiline among indianmdr-tb patients in clinical settings; Authorerea. 2021;24:34-40.

21. Rajput SJ, Vanavi PJ. Evaluation of physicochemical stability and degradation kinetics of bedaquiline in hydrolytic solutions of different pH; Future Journal of Pharmaceutical Sciences. 2021;7(1):88.

22. Kumar AKH, Sudha V, Vijaya Kumar A, Padmapriyadarshini C. Simultaneous method for the estimation of bedaquiline and delamanid in human plasma using high-performance liquid chromatography. International Journal of Pharmacy and Pharmaceutical Sciences. 2021;13(6):36–40.

23. Code Q2 (R1) -Text on validation of analytical procedures: Text and methodology current step 4 version, 2005, ICH Harmonised Tripartite Guideline.