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Validation of UV spectrophotometric method for estimation of bedaquiline fumarate in bulk and pharmaceutical formulations

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ABSTRACT: The FDA-approved diaryl-quinoline anti-mycobacterial drug called bedaquiline fumarate is used to treat pulmonary multi drug resistant tuberculosis. It inhibits proton pump of mycobacterial ATP synthase, an enzyme necessary for production of energy in mycobacterium tuberculosis bacteria. According to ICH Q2(R1) guideline, this research presents simple, rapid, reproducible and cost-effective UV spectrophotometric method for estimation of bedaquiline fumarate in bulk and pharmaceutical formulations. Phosphate buffer pH 7.4 was selected as solvent for the measurement of λ_{max} at 200nm-400nm wavelength range and further investigated for Linearity, Accuracy, Ruggedness, Intraday and Interday Precision, Limit of quantification (LOQ) and limit of detection (LOD). The λ_{max} in phosphate buffer pH 7.4 was found to be 285.88nm and the method was discovered to be linear over the range of 10µg/ml-70µg/ml with R^2 value of 0.9974. After the concentration spikes of 80%, 100%, and 120% in the accuracy study, the mean %recovery was determined to be 101.18%, 100.95%, and 100.60%, respectively, and %RSD was less than 2.0%. For the 10µg/ml sample solution, there was no appreciable difference in the data from two independent analysts. The suggested method was therefore thought to be reproducible and rugged. Intraday and interday precision were demonstrated with less than 2.0%RSD. The Limit of quantification (LOQ) was determined to be 3.884µg/ml and the limit of detection (LOD) to be 1.282µg/ml. The assay of bedaquiline fumarate didn't interfere by the excipients used in the formulation of inhaled dry powder of bedaquiline fumarate loaded nano structured lipid carrier (NLC) capsules 50mg. This method is useful for routine analysis of bedaquiline fumarate in bulk and pharmaceutical formulations.

KEYWORDS: Bedaquiline Fumarate; UV Spectrophotometry; bedaquiline fumarate loaded NLC; DPI; ICH Q2(R1) Guideline.

1. INTRODUCTION

After 40 years, FDA approved a new molecule i.e. bedaquiline fumarate (BDQ) for the treatment of pulmonary multi drug resistant tuberculosis (MDR-TB)[1,2]. It is diaryl-quinoline anti-mycobacterial drug chemically known as (1R,2S)-1-(6-bromo-2-methoxyquinolin-3-yl)-4-(dimethylamino)-2-naphthalen-1-yl-1-phenylbutan-2-ol;(E)-but-2-enedioic acid [3]. It inhibits proton pump of mycobacterial ATP synthase, an enzyme necessary for production of energy in mycobacterium tuberculosis bacteria[1,2,3]. BDQ is a BCS class II drug having low solubility and high permeability[4]. Based on literature survey, BDQ is insoluble in water and over a wide range of pH[3,4].

Due to its intrinsic simplicity, low cost, and widespread availability in quality control laboratories, UV spectrophotometry is the most practical analytical technology for routine examination[5,6]. By carrying out methodical tests in the phosphate buffer pH 7.4 for the validation of BDQ in bulk and pharmaceutical formulations, an effort has been made to develop a simple, rapid, reproducible and cost-effective UV spectrophotometric method.

2. RESULTS AND DISCUSSION

2.1. Determination of λ_{max}

The λ_{max} of BDQ in phosphate buffer pH 7.4 was found to be 285.88nm. Spectra of 50µg/ml BDQ sample solution is mentioned in Figure 1.

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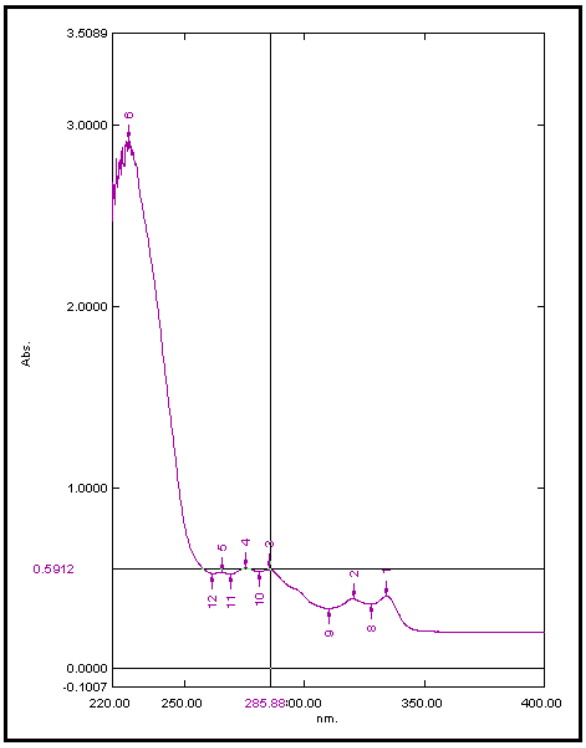

Figure 1. Spectra of 50µg/ml sample solution of BDQ in Phosphate buffer pH 7.4

2.2. Linearity

The linearity of BDQ was found to be in range of 10µg/ml-70µg/ml with correlation coefficient 0.9974 **[Figure 2]**. All the quantitative parameters are mentioned in Table 1. The linearity of the calibration curve **[Figure 2]** demonstrated that the suggested analytical method can be utilized successfully to analyze BDQ in pharmaceutical formulations without any interference of excipients.

Table 1. Standard calibration curve of BDQ in phosphate buffer pH 7.4.

Concentration (µg/ml)	Mean absorbance ± S.D (n=3)
10	0.0853 ± 0.001
20	0.2255 ± 0.003
30	0.3689 ± 0.001
40	0.4993 ± 0.007
50	0.5912 ± 0.002
60	0.7372 ± 0.001
70	0.8506 ± 0.008

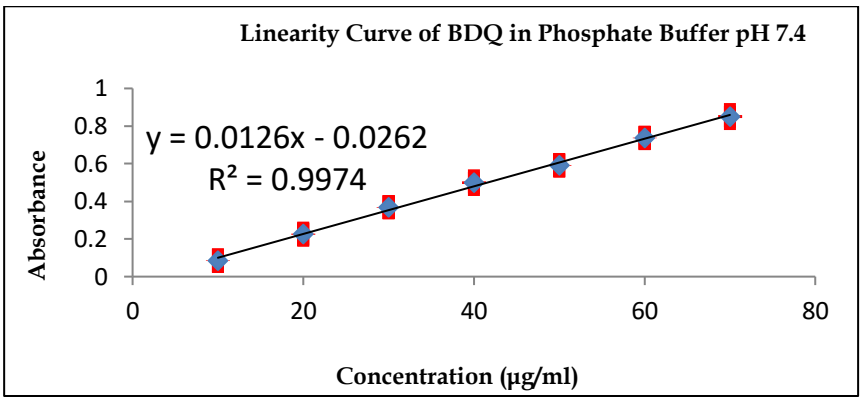

Figure 2. Linearity curve of BDQ in phosphate buffer pH 7.4 (10µg/ml-70µg/ml)

2.3. Determination of precision

2.3.1 Intraday precision

For intraday precision, the %RSD was found to be in limit (NMT 2.0%) for 10µg/ml, 40µg/ml and 70µg/ml. The outcomes are listed in Table 2.

Table 2. Intraday precision

Concentration of BDQ (µg/ml)	Absorbance				SD	%RSD (Limit: NMT 2.0%)
	1	2	3	Average		
10	0.0852	0.0849	0.0843	0.0848	0.0005	0.5404
40	0.4982	0.4984	0.4995	0.4987	0.0007	0.1404
70	0.8507	0.8509	0.8512	0.8509	0.0003	0.0296

2.3.2 Interday precision

For interday precision, the %RSD was found to be in limit (NMT 2.0%) for 10µg/ml, 40µg/ml and 70µg/ml. The outcomes are listed in Table 3.

Table 3. Interday precision

Concentration of BDQ (µg/ml)	Absorbance				SD	%RSD (Limit: NMT 2.0%)
	1	2	3	Average		
10	0.0850	0.0855	0.0846	0.0850	0.0005	0.5303
40	0.4978	0.4985	0.4991	0.4985	0.0007	0.1305
70	0.8512	0.8516	0.8514	0.8510	0.0002	0.0235

2.4. Accuracy

%Recovery study was performed at three distinct levels, such as 80%, 100% and 120% of spike concentration of 10µg/ml of BDQ sample solution. The mean %recovery was found to be 101.18%, 100.95% and 100.60% respectively and %RSD was found to be in limit (NMT 2.0%). The outcomes are listed in Table 4.

Table 4. Accuracy study

% of spike concentration	Concentration of standard spike (µg/ml)	Concentration of sample (µg/ml)	Total concentration taken (µg/ml)	Total found concentration (µg/ml)	% recovery	Mean % recovery	SD	%RSD (Limit: NMT 2.0%)
80%	8	10	18	18.46	102.56	101.18	1.38895	1.37281
			18	18.21	101.19			
			18	17.96	99.78			
100%	10	10	20	19.86	99.29	100.95	1.44828	1.43461
			20	20.33	101.67			
			20	20.38	101.90			
120%	12	10	22	21.85	99.31	100.60	1.13182	1.12506
			22	22.23	101.05			
			22	22.32	101.44			

2.5. Ruggedness

There was no discernible variation in the data from two independent analysts for 10µg/ml sample solution, the results obtained and mentioned in Table 5 were determined to be reproducible. As a result, the suggested technique was deemed to be rugged.

Table 5. Ruggedness (Sample solution: 10µg/ml)

Analyst	Absorbance				SD	%RSD (Limit: NMT 2.0%)
	1	2	3	Average		
Analyst 1	0.0851	0.0853	0.0854	0.0853	0.0002	0.1792
Analyst 2	0.0856	0.0854	0.0852	0.0854	0.0002	0.2342

2.6. Limit of detection (LOD) and Limit of quantification (LOQ)

The sensitivity of the method was evaluated by calculating the LOD and LOQ. The LOD and LOQ for BDQ in phosphate buffer pH 7.4 were discovered to be 1.282µg/ml and 3.884µg/ml respectively.

2.7. Assay of inhaled dry powder of BDQ loaded nano structured lipid carrier (NLC) capsules 50mg

The amount of BDQ in the DPI capsules of BDQ loaded NLC was estimated with the established analytical method and average of %drug recovered was found to be 99.98% as shown in Table 6, hence indicating that the method can be effectively applied to analyse BDQ in different pharmaceutical formulations.

Table 6. Assay of DPI capsules of BDQ loaded NLC

Formulation	Label claim (mg)	Amount of BDQ detected (mg) (n=3)	%Drug detected	Average of %drug detected	SD	% RSD (Limit: NMT 2.0%)
Inhaled dry powder of BDQ loaded NLC capsules 50mg	60.45mg BDQ eq. to 50mg Bedaquiline	60.43mg 60.45mg 60.44mg	99.97% 100.00% 99.98%	99.98%	0.017	0.017

3. CONCLUSION

The development of UV spectrophotometric technique for the determination of BDQ in bulk and pharmaceutical formulations is simple, rapid, reproducible, economical, successful and developed as per ICH Q2(R1) guideline. All of the reagents employed in the investigation were affordable, reliable, and easily accessible in the analytical laboratory. The approach is simple to use and appropriate for regular analysis in quality control laboratories.

4. MATERIALS AND METHODS

4.1. Sample, Chemical and Reagent

BDQ was received as a gift sample from dishman carbogen amcis ltd., india. Analytical-grade reagents were utilized throughout the study. Di-sodium hydrogen phosphate heptahydrate ($Na_2HPO_4.7H_2O$), sodium phosphate monobasic monohydrate ($NaH_2PO_4.H_2O$), hydrochloric acid and sodium hydroxide were procured from sigma-aldrich chemicals pvt. ltd., india. Sodium lauryl sulphate (SLS) was procured from anmol chemicals pvt. ltd., india.

4.2. Instrumentation

UV analysis was carried out at 200nm-400nm wavelength range by double beam UV spectrophotometer (Model: UV-1800, Make: Shimadzu, Japan) with a UV probe 4.21 software by using 1 cm quartz cuvettes at 25°C. pH of the solutions was measured by a digital pH meter (Model: S-400, Make: Mettler-Toledo India Pvt. Ltd, India).

4.3. Reagent

4.3.1. Preparation of 0.2M hydrochloric acid solution (1000ml)

16.6ml of concentrated hydrochloric acid was added gradually to 800ml of distilled water and finally made upto 1000ml with distilled water to get a 1000ml 0.2M hydrochloric acid solution.

4.3.2. Preparation of 0.2M sodium hydroxide solution (1000ml)

8g of NaOH pellets were dissolved in 800ml of distilled water until clear solution was obtained and made upto 1000ml with distilled water to get a 1000ml 0.2M sodium hydroxide solution.

4.3.3. Preparation of phosphate Buffer pH 7.4 (1000ml) [7]

20.214g of Di-Sodium hydrogen phosphate heptahydrate ($Na_2HPO_4.7H_2O$) and 3.394g of sodium phosphate monobasic monohydrate ($NaH_2PO_4.H_2O$) were dissolved in 800ml of distilled water until it became a clear solution. The pH was measured and if required, it was adjusted with 0.2M hydrochloric acid solution and 0.2M sodium hydroxide solution. The volume was made upto 1000ml with distilled water and filtered to obtain 1000ml phosphate Buffer pH 7.4.

4.4. Preparation of stock and sample solutions

4.4.1. Preparation of stock solutions

Stock solution-1:

100mg of BDQ and 500mg of SLS were weighed accurately and dissolved in 80ml phosphate buffer pH 7.4. The volumetric flask was kept in ultra-sonication bath until it became a clear solution. It was made upto 100ml with phosphate buffer pH 7.4 to obtain 1000µg/ml stock solution-1.

Stock solution-2:

10ml of stock-1 solution was diluted upto 100ml with phosphate buffer pH 7.4 to obtain 100µg/ml stock solution-2.

4.4.2. Preparation of sample solutions

1ml, 2ml, 3ml, 4ml, 5ml, 6ml and 7ml of stock solution-2 were diluted upto 10ml with phosphate buffer pH 7.4 to obtain 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml, 60µg/ml and 70µg/ml solutions respectively.

4.5. Validation procedure [8-11]

4.5.1. Determination of λmax

50µg/ml of BDQ sample solution was scanned in double beam UV spectrophotometer in the range of 200nm-400nm by using phosphate buffer pH 7.4 and 500mg SLS mixture solution was used as blank. λmax was measured from the spectra obtained.

4.5.2. Determination of linearity

Linear regression analysis was performed by measuring the absorbance of sample solution of 10µg/ml-70µg/ml at λmax=285.88nm against phosphate buffer pH 7.4 and 500mg SLS mixture solution as blank. The calibration curve was plotted for concentration (µg/ml) Vs. absorbance (nm) and correlation coefficient with regression line equation for BDQ was determined.

4.5.3. Determination of precision

Intraday precision:

Intraday precision was determined by measuring the absorbance of 10µg/ml, 40µg/ml and 70µg/ml sample solution at three different time points of the same day and %RSD was measured.

Interday precision:

Interday precision was determined by measuring the absorbance of 10µg/ml, 40µg/ml and 70µg/ml sample solution at three different time points on different days and %RSD was measured.

4.5.4. Accuracy

Accuracy of analytical method was determined by %recovery study performed at three distinct levels, such as 80%, 100% and 120% of spike concentration of 10µg/ml of BDQ sample solution. %recovery was calculated by following equation.

4.5.5. Ruggedness

Ruggedness of analytical method was determined by performing the same analytical procedure of assay under same conditions with same instrument by two different analysts on different days for 10µg/ml sample solution. Reproducibility was assessed for the results.

4.5.6. Limit of detection (LOD) and limit of quantification (LOQ)

According to the ICH Q2(R1) guideline, the LOD of an analytical method is the lowest concentration of analyte in the sample, which can be detected but not necessarily quantitated while the LOQ of an analytical method is the lowest concentration of analyte in the sample that can be quantitatively measured with necessary accuracy and precision. LOD and LOQ were calculated by following equations.

$$\mathrm{LOD} = \frac{3.3\sigma}{S}$$

$$\mathrm{LOQ} = \frac{10\sigma}{S}$$

Where, σ = Standard deviation of the response, S= Slope

4.5.7. Determination of assay of inhaled dry powder of BDQ loaded nano structured lipid carrier (NLC) capsules 50mg

60.45mg BDQ (60.45mg BDQ Equivalent to 50mg of Bedaquiline) equivalent powder from the DPI capsule and 500mg of SLS were weighed. They were dissolved in 80ml of phosphate buffer pH 7.4 and the volumetric flask was kept in ultra-sonication bath until it became a clear solution. It was made upto 100ml with phosphate buffer pH 7.4 to obtain 604.5µg/ml stock solution. The solution was filtered and 10ml of it was diluted upto 100ml with phosphate buffer pH 7.4 to obtain 60.45µg/ml solution. The absorbance was measured at λmax=285.88nm. The concentration of BDQ was measured by regression line equation obtained from linearity study.

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