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VALIDATED SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATION OF BEDAQUILINE IN BULK AND TABLET DOSAGE FORM

Chethan Gowda S.*[1], Jose Gnana Babu C.*[2] and Sowmya H. G.*[3]

*[1]2nd Year M Pharma, Student of Department of Pharmaceutical Analysis Bharathi College of Pharmacy, Mandya, Karnataka, India-571422.

*[2]Professor and HOD of Department of Pharmaceutical Analysis Bharathi College of Pharmacy, Mandya, Karnataka, India-571422.

*[3]Assistant Professor of Department of Pharmaceutical Analysis Bharathi College of Pharmacy, Mandya, Karnataka, India-571422.

***Corresponding Author: Chethan Gowda S.**

2nd Year M Pharma, Student of Department of Pharmaceutical Analysis Bharathi College of Pharmacy, Mandya, Karnataka, India-571422.

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ABSTRACT

Simple, precise and accurate area under curve spectroscopic method has been developed and validated for the estimation of Bedaquiline in bulk and pharmaceutical dosage form. The drug shows maximum absorption (λ_{max}) at 226nm in Methanol solution and Area under Curve [AUC] in absorption spectra were measured between the wavelength range 221 to 231nm which obeys Beer's law in the concentration range of 0.5-2.5 µg/ml. The linearity study was carried out and regression coefficient was found to be 0.9999 and it has showed good linearity, precision during this concentration range. The % recovery was found to be 96.14-99.15. The LOD and LOQ were found to be 0.0197 and 0.0598µg/ml. The percentage relative standard deviation was found to be less than 2. According to ICH guidelines the method have been validated for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed and validated method can be successfully applied for routine quantitation of Bedaquiline in bulk and pharmaceutical dosage form.

KEYWORDS: Bedaquiline, Area under curve spectroscopy, validation, pharmaceutical formulations.

INTRODUCTION

Bedaquiline is a new antitubercular drug belonging to the di arylquinoline class that efficiently inhibits the adenosine triphosphate synthase enzyme of Mycobacterium tuberculosis. Bedaquiline offers a new mechanism of anti-TB action by specifically inhibiting mycobacterial adenosine triphosphate (ATP) synthase.[1]

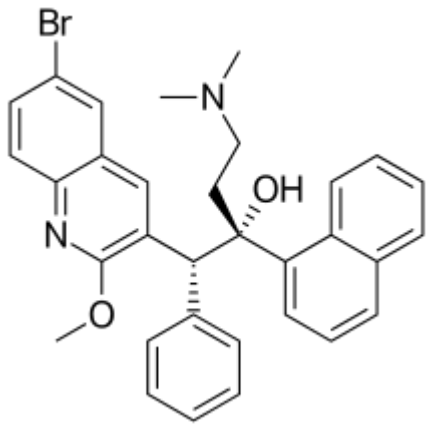

Fig. 1: Chemical structure of Bedaquiline.

Bedaquiline is chemically known as (1R,2S)-1-(6-Bromo-2-methoxy-3-quinolyl)-4-dimethyla-mino-2-(1-naphthyl)-1-phenylbutan-2-ol with a molecular formula of $C_{32}H_{31}BrN_2O_2$ and molecular weight of 555.516 $g \cdot mol^{-1}$. Bedaquiline drug substance is White Crystalline powder and it is soluble in organic solvents such as ethanol and dimethyl formamide.

MATERIALS AND METHODS

Instrument: Ultraviolent-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken on weighing balance.

Chemicals: Bedaquiline pure drug was obtained as a gift sample from Recipharma pharmaservices pvt Ltd,T Begur, Bengaluru and its pharmaceutical dosage form Bedaquiline 20 tablet labelled claim 100mg from local pharmacy manufactured by Synokem Pharma India Ltd.

Solvent: Methanol used as a solvent.

Selection of analytical wavelength: Appropriate dilutions of Bedaquiline were prepared from standard

stock solution and using spectrophotometer solution was scanned in the wavelength range 200-400nm. Area under Curve [AUC] in absorption spectra were measured between the wavelength range 221 to 231nm as the wavelength for detection (Fig-2).

Preparation of standard stock solution: 100mg of Bedaquiline was weighed accurately and transferred in to 10ml volumetric flask and diluted in Methanol up to mark. From this, the solution was further diluted into 100µg/ml and pipetted out 0.5, 1.0, 1.5, 2.0, and 2.5ml, into 10ml individual volumetric flask and diluted in Methanol up to the mark and this gives 0.5, 1.0, 1.5, 2.0, and 2.5µg/ml concentration.

Preparation of sample solution: 10 tablets of Bedaquiline marketed formulations was weighed and powdered. A quantity of tablet powder equivalent to 100mg of Bedaquiline was transferred into a 100ml of volumetric flask then it was diluted with Methanol and made up to the mark.

METHOD AND VALIDATION: The method was validated according to ICH guidelines.

RESULTS AND DISCUSSION

Method: Area under curve spectroscopy.

Linearity: The linearity of an analytical method is its dimensions to show the test results that are directly proportional to the concentration of the analyte in the sample within the range. The linearity was established in the range of 0.5-2.5µg/ml and Area under Curve [AUC] in absorption spectra were measured between the wavelength of 221 to 231nm as absorbance values are shown in table-1 (Fig-3). The calibration curve was prepared by plotting graph against the concentration and absorbance and therefore the graph shown in (Fig-4). Statistical variables like slope, intercept, regression equation, correlation coefficient and Sandell's sensitivity were determined. (table-2).

Precision: The precision of an analytical method expresses the closeness of a series of individual analyte measurements obtained from multiple sampling of the equivalent sample. Precision is determined by intra-day and inter-day study. Intra-day precision was determined by analysing the same concentration for six times in a same day. Inter-day precision was determined by analysing the same concentration daily for six days. (table-3).

Accuracy: The accuracy of an analytical method says that closeness of test results obtained by that method to the true value. To assess the accuracy of the developed method, recovery studies were carried out at three different levels as 50%, 100% and 150%. In which the formulation concentration hold constant and varied pure drug concentration. (table-4).

Ruggedness: The ruggedness is defined as the reproducibility of results when the method is performed under the different in conditions. This includes distinct analyst, laboratories, instruments, temperature etc. Ruggedness was determined between different analyst; the value of %RSD was found to be less than 2. (table-5).

LOD and LOQ: The limit of detection of individual analytical method is the smallest amount of analyte in a sample which can be reliably detected by the analytical method. The limit of quantitation of individual analytical procedure is the smallest amount of analyte in a sample which can be quantitatively determined. LOD and LOQ was calculated by using following formula.

LOD = 3.3(SD)/S and LOQ = 3(LOD)

LOD and LOQ value of were found Bedaquiline be 0.0197and 0.0598µg/ml.

TABLES

Table 1: Results of calibration curve at 221-231nm by Area under curve method.

Sl no	Concentration in µg/ml	Absorbance ± Standard deviation*
1	0	0
2	0.5	0.105±0.00211
3	1.0	0.202±0.00115
4	1.5	0.298±0.00141
5	2.0	0.397±0.00094
6	2.5	0.495±0.00095

*Average of six determinations.

Table 2: Regression parameter for Bedaquiline at 221-231nm by Area under curve method.

Regression parameter	Results
Range(µg/ml)	0.5-2.5
Detection Wavelengths (nm)	221/231
Regression Equation	Y= 0.197x+0.0033
Slope(b)	0.197
Intercept(a)	0.0033

Correlation coefficient(r^2)	0.9999
Sandell's equation	0.0050
Limit of detection(µg/ml)	0.0197
Limit of quantitation(µg/ml)	0.0598

Table 3: Determination of precision results for Bedaquiline at 221-231nm by Area under curve method.

Concentration (µg/ml)	Intra-day Absorbance ±Standard deviation*	%RSD**	Inter-day Absorbance ±Standard deviation*	%RSD**
0.5	0.105±0.00129	1.22	0.105±0.00134	1.27
1.0	0.205±0.00150	0.73	0.206±0.00189	0.91
1.5	0.294±0.00149	0.50	0.294±0.00124	0.42
2.0	0.394±0.00146	0.37	0.395±0.00110	0.27
2.5	0.494±0.00110	0.22	0.495±0.00134	0.27

*****Average of six determinations, **percentage relative standard deviation.

Table 4: Determination of Accuracy results for Bedaquiline at 221-231nm by Area under curve method.

Spiked Levels	Amount of Sample (µg/ml)	Amount of Standard (µg/ml)	Amount Recovered	% Recovery ±Standard deviation*	%RSD**
50	1.5	0.75	2.16	96.14 ±0.3309	0.344
100	1.5	1.5	2.94	98.27 ±0.3550	0.361
150	1.5	2.25	3.71	99.15 ±0.3246	0.327

*****Average of six determinations, **percentage relative standard deviation.

Table 5: Determination of Ruggedness results for Bedaquiline at 221-231nm by Area under curve method.

Analysts	Analyst 1	Analyst 2
Mean absorbance	0.295	0.294
±Standard deviation*	0.001291	0.001258
%RSD	0.437	0.427

*Average of six determinations, **percentage relative standard deviation.

FIGURES

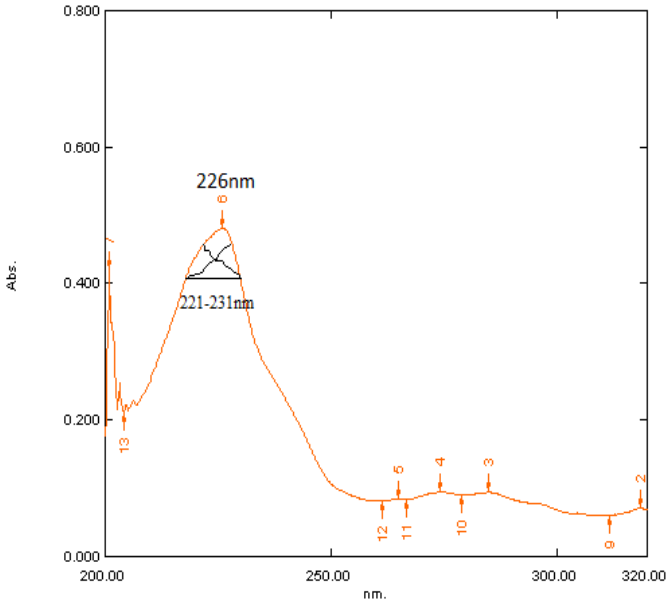

Fig. 2: Area under curve spectrum of Bedaquiline at 221-231nm.

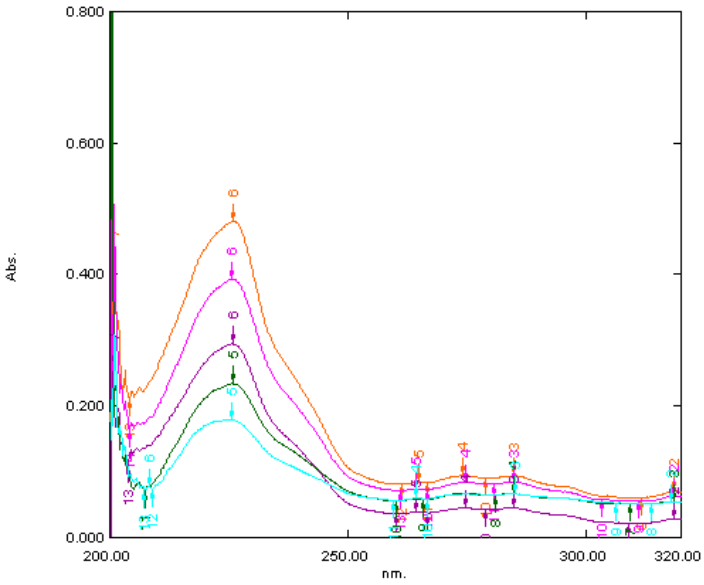

Fig. 3: Area under curve overlain spectra of Bedaquiline showing absorbance at 221-231nm.

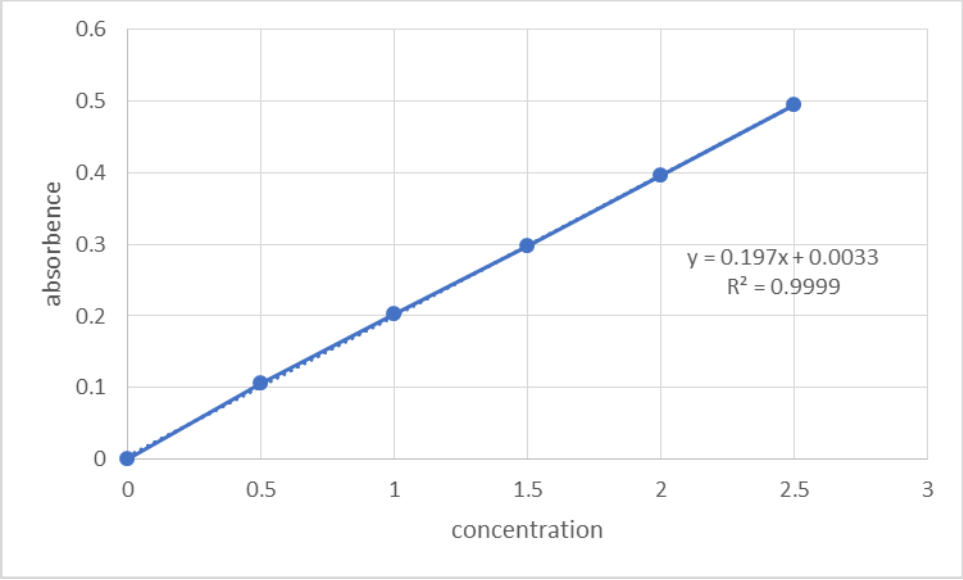

Fig.4: Calibration curve of Bedaquiline at 221-231nm by Area under curve.

CONCLUSION

As per ICH guidelines, the developed analytical method meets the acceptance criteria. It was concluded that the method is simple, specific, accurate, economical, sensitive and can be used for routine analysis of Bedaquiline in bulk drug and in pharmaceutical dosage forms.

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