Research Article **CODEN: AJPAD7** **ISSN: 2321 - 0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

https://doi.org/10.36673/AJPAMC.2022.v10.i01.A02

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

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ABSTRACT

Simple, precise and accurate zero order derivative spectroscopic method has been developed and validated for the estimation of Bedaquiline (sirturo 100mg) in bulk and pharmaceutical dosage form. The drug shows maximum absorption (λ max) at 226nm in Methanol solution and obeys Beer's law in the concentration range of 0.5-2.5µg/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 96.88-99.59. The LOD and LOQ were found to be 0.0254 and 0.0770µg/ml. The percentage relative standard deviation is found to be less than 2. As per ICH guidelines the technique has been validated for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed and validated method can be successfully applied for routine quantification of Bedaquiline in bulk and pharmaceutical dosage form.

KEYWORDS

Bedaquiline, Zero order derivative spectroscopy, Validation and Pharmaceutical formulations.

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INTRODUCTION

Bedaquiline is a new antitubercular drug belonging to the di-aryl-quinoline 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].

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 a molecular weight of 555.516g·mol^{-1}. Bedaquiline drug substance is

White Crystalline powder and it is soluble in organic solvents such as ethanol and dimethyl formamide.

MATERIAL AND METHODS

Instrument

UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) along with UV probe software. All weights were taken in analytical balance.

Chemicals

Bedaquilinepure drug was obtained as a gift sample from Recipharma Pharma Services 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. The absorption spectra obtained and show maximum absorbance at 226nm, as the wavelength for detection (Figure No.2).

Preparation of standard stock solution

100mg of Bedaquiline was weighed accurately and transferred in to 100ml volumetric flask and diluted in Methanolup 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 Methanolup 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 ofBedaquiline was transferred into a 100ml of volumetric flask then it was diluted withMethanol and made up to the mark.

Method and validation

The method was validated according to the ICH guidelines.

RESULTS AND DISCUSSION

Method: Zero order derivative spectroscopy

Linearity

The linearity of an analytical method is its dimension 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 was measured at 226nm and absorbance values are shown in Table No.1. The calibration curve was prepared by plotting graph against the concentration and absorbance and therefore the graph shown in Figure No.3. Statistical variables like slope, intercept, regression equation, correlation coefficient and sandell's sensitivity were determined. (Table No.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 was established by intra-day and inter-day studies. 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 No.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 levelsas 50%, 100% and 150%. In which the formulation concentration holds it constant and varied pure drug concentration. (Table No.4).

Ruggedness

The ruggedness is defined as the reliability of results when the method is performed under the variation in conditions. This includes distinct analyst, laboratories, instruments, temperature etc.Ruggedness was determined between distinct analyst, the value of %RSD was found to be less than 2. (Table No.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 Bedaquiline were found be 0.0254 and 0.0770µg/ml.

Table No.1: Results of calibration curve at 226nm by zero order spectroscopy

S.No	Concentration in µg/ml	Absorbance ± Standard deviation*
1	0	0
2	0.5	0.104±0.00249
3	1.0	0.201±0.00280
4	1.5	0.298±0.00601
5	2.0	0.396±0.00689
6	2.5	0.495±0.00647

*Average of six determinations

Table No.2: Regression parameter for Bedaquiline by zero order spectroscopy

S.No	Regression parameter	Results
1	Range (µg/ml)	0.5-2.5
2	λ_{max}(nm)	226
3	Regression Equation	Y= 0.197x+0.0027
4	Slope (b)	0.197
5	Intercept (a)	0.0027
6	Correlation Coefficient (r^2)	0.9999
7	Sandell's equation	0.0050
8	Limit of detection (µg/ml)	0.0254
9	Limit of quantitation (µg/ml)	0.0770

Table No.3: Determination of precision results for Bedaquiline at 226nm by zero order spectroscopy

S.No	Concentration (µg/ml)	Intra-day Absorbance ±Standard deviation*	%RSD**	Inter-day Absorbance ±Standard deviation*	%RSD**
1	0.5	0.106±0.00203	1.91	0.106±0.00195	1.83
2	1.0	0.204±0.00221	1.08	0.216±0.00177	0.81
3	1.5	0.299±0.00561	1.87	0.299±0.00501	1.67
4	2.0	0.396±0.00608	1.53	0.398±0.00706	1.77
5	2.5	0.497±0.00119	0.23	0.491±0.00476	0.96

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

Table No.4: Determination of Accuracy results for Bedaquiline at 226nm by Zero order spectroscopy

S.No	Spiked Levels	Amount of Sample (µg/ml)	Amount of Standard (µg/ml)	Amount Recovered	% Recovery ±Standard deviation*	%RSD**
1	50	1.5	0.75	2.18	96.88 ±0.571	0.589
2	100	1.5	1.5	2.95	98.60 ±0.447	0.453
3	150	1.5	2.25	3.73	99.59 ±0.255	0.256

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

Table No.5: Determination of Ruggedness results for Bedaquiline at 226nm by Zero order spectroscopy

S.No	Analysts	Analyst 1	Analyst 2
1	Mean absorbance	0.296	0.295
2	±Standard deviation*	0.00170	0.00291
3	%RSD	0.574	0.986

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

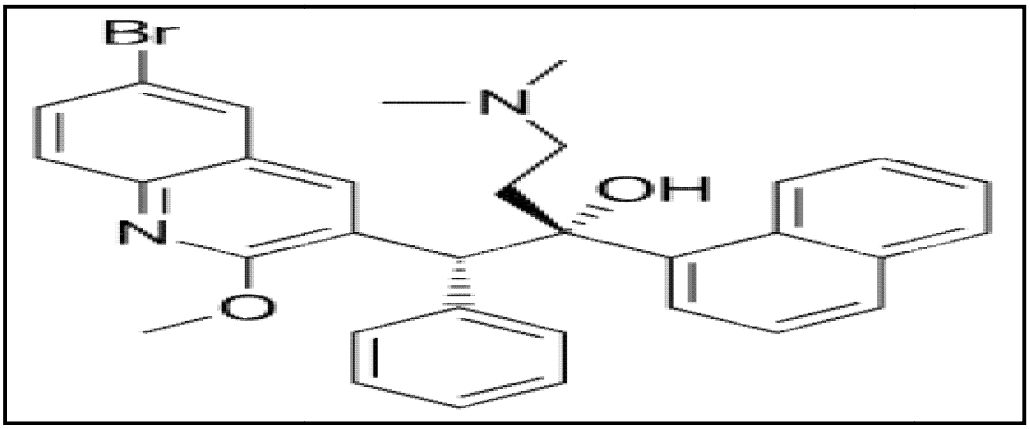

Figure No.1: Chemical structure of Bedaquiline

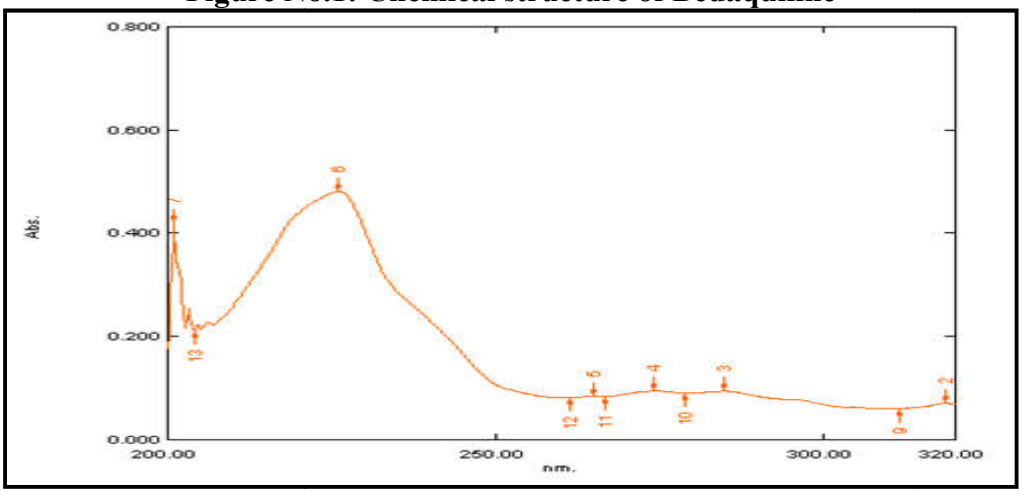

Figure No.2: Zero order spectrum of Bedaquiline at 226nm

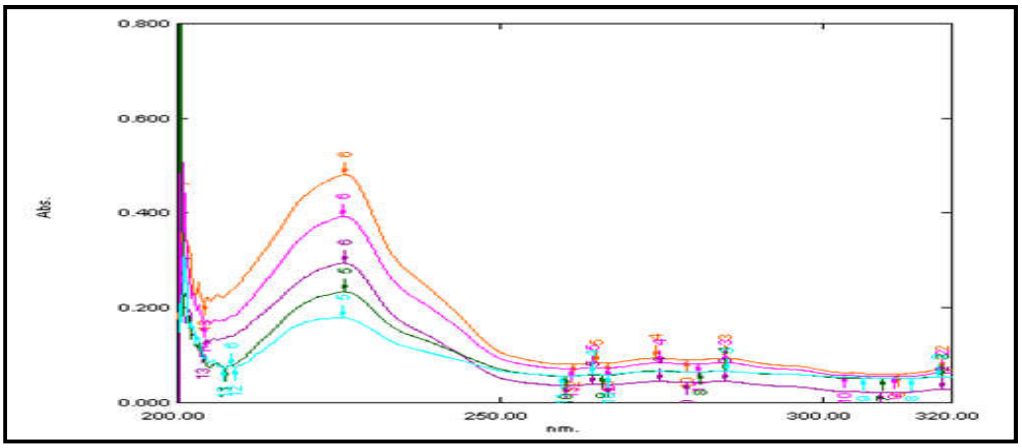

Figure No.3: Zero order overlain spectra of Bedaquiline showing absorbance at 226nm

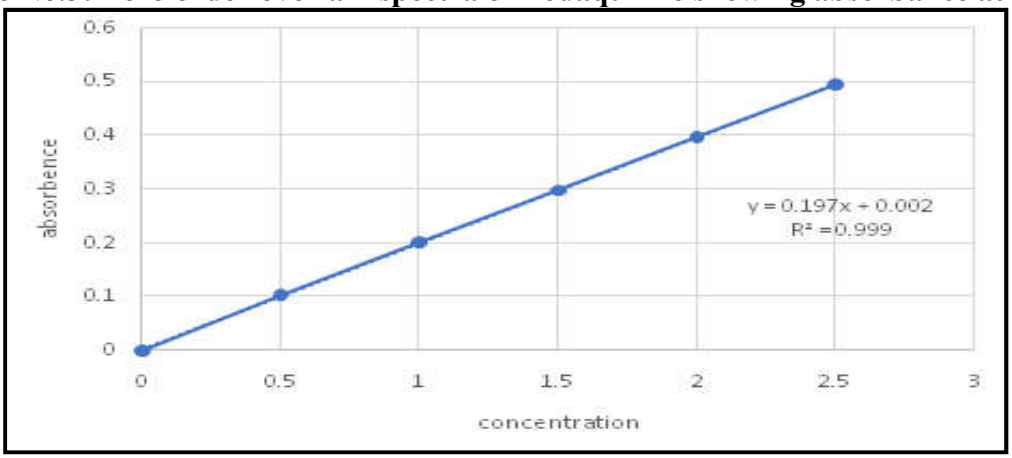

Figure No.4: Calibration curve of Bedaquiline by zero order spectroscopy

CONCLUSION

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

ACKNOWLEDGEMENT

We authors wish to thanks to our management, Principal of Pharmacy College for providing all facilities in the college.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Chethan Gowda S *et al*. Validated spectrophotometric method for the quantitation of Bedaquiline in bulk and tablet dosage form, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry,* 10(1), 2022, 7-12.