Research Article **ISSN: 2395 – 5597**

International Journal of Engineering and Robot Technology

Journal home page: www.ijerobot.com

https://doi.org/10.36673/IJEROBOT.2021.v08.i02.A10

VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF BEDAQUILINE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A new sensitive, specific, linear, precise and accurate RP-HPLC method was developed and validated for estimation of Bedaquiline in Bulk and Tablet dosage form. An isocratic, reversed phase HPLC method was developed. Shimadzu shim pack C18(250mm x 4.5µm, x 5µ) column. Shimadzu Prominence-I LC-2030C plus equipped with Auto sampler as the instrument model. Mobile phase consists of mixture of methanol and 10Mm ammonium acetate buffer in the ratio (85:15 v/v) at a flow rate of 0.5ml /min with injection volume of 20µL. UV detection was performed at 226nm. The Linearity was established for Bedaquiline in the range of 2-64µg/ml with correlation coefficient of 0.9998. LOD and LOQ were found to be 0.29477µg/ml and 0.9825µg/ml respectively. Retention time of Bedaquiline were found to be 2.9mins. The % Recovery was found to be 98.81-99.23 and %RSD was found with in ± 2. The method has been validated according to ICH guidelines for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed validated method was successfully applied for reliable quantification of Bedaquiline in bulk and pharmaceutical dosage form.

KEYWORDS

Bedaquiline, RP- HPLC, 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 amolecular 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 form amide.

Literature survey revealed that there were few analytical methods have been reported for the determination of the Bedaquiline in pure drug and pharmaceutical dosage form by using UV-Spectrophotometric[2], and RP-HPLC[3-9] so far.

The aim of the present work is to develop and validate a novel, rapid, precise and specific Area under curve UV spectrophotometric method for estimation of Bedaquiline in bulk and tablet dosage form.

MATERIAL AND METHODS

Material and reagents

The Bedaquiline was obtained as a gift sample from the pharmaceutical industry and sirturo (100mg) tablet obtained from Pharmacy store. Methanol, 10Mm Ammonium acetate buffer were obtained Bharathi College of pharmacy, Bharathinagara, KM Doddi, Maddur Taluk, Mandya District, India. All chemicals used are of HPLC grade. Distilled water was used throughout the experiment.

Instrumentation

Chromatographic separation was performed on a Shimadzu Prominence-i LC-2030 plus equipped with Auto sampler comprising a variable wavelength programmable UV detector. Shimadzu shim pack C18 (250mm x 4.5µm x 5µ) column is used.

Preparation of mobile phase

The mobile phase was prepared by mixing methanol and 10Mm ammonium acetate buffer (pH4.5) (85:15 v/v).

Preparation of buffer

Dissolve 13.61g of sodium acetate trihydrate in 800ml of Distilled water and mix using a magnetic stirrer with the pH meter to check the pH. Adjust the pH by adding 10mM acetic acid prepared from acetic acid 100% (17.4M) until pH 4. Then transfer into 1L volumetric flask and adjust 1000ml with Distilled water.

Preparation of sample Standard Solution

Sirturo 10 tablets, marketed formulation of Bedaquiline containing 100mg of the drug was calculated for the % assay of the Bedaquiline in the formulation. A concentrated solution of 10-1µg/mL was obtained using a powder made up of 10mg. The filtered solution was employed in the HPLC apparatus to perform the percentage assay of Bedaquiline.

Preparation of Standard solution

A standard stock solution of Bedaquiline (1000µg/ml) was prepared individually by dissolving accurately weighed, 10mg of drug in 10ml volumetric flask in some quantity of mobile phase and final dilution up to the mark with the mobile phase. From this stock solution, 1ml aliquot was transferred and diluted up to the mark with diluents in 10ml volumetric flask to obtain a final concentration of 100µg/ml.

System suitability requirements from stock and standard solutions

a) Tailing factor: NMT 2.0

b) Theoretical Plates: NLT 2000

RESULTS AND DISCUSSION

Validation of the proposed method

The proposed method was validated as per ICH guidelines[10-12]. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability.

Specificity

The selectivity of the analytical method was evaluated by the analysis of a solution containing Bedaquiline. There was no interferences observed at retention time of Bedaquiline from diluent solution. The results for the same were summarized in Table No.2.

Linearity

The linearity of the response of the drug was verified at six concentration levels, ranging from 2-64µg/ml of Bedaquiline in each linearity level were prepared. 20µl of each concentration was injected into the HPLC system. The response was read at 226nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were presented in Table No.3.

Precision

Precision of the method was performed as intraday precision, Inter day precision. To study the intraday precision, six replicate standard solutions (8µg/ml) of Bedaquiline were injected. % RSD was calculated and it was found to be 1.102 and interday precision done same as intraday, six replicate standard solutions (8µg/ml) of Bedaquiline were injected. % RSD was calculated and it was found to be 1.34which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table No.4.

Accuracy

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo. The recovery was performed at three levels, 50, 100 and 150% of the label claim of the tablet (100mg of Bedaquiline). The recovery values for Bedaquiline ranged from 98.81 to 99.23%. The average recoveries of three levels of Bedaquiline were found to be 98.81 to 99.23%. The results are shown in the Table No.5.

Limit of detection and Limit of quantification

The limit of detection is an analytical method is the smallest amount of analyte in a sample which can be reliable detected by the analytical method. The limit of quantitation is an individual analytical procedure is the smallest amount of the analyte in sample which can be quantitatively determined. LOD and LOQ were calculated using formula LOD = 3.3(SD)/S and LOQ = 10(SD)/S. Results were shown in Table No.6.

Ruggedness

The ruggedness of test method was demonstrated by carrying out precision study in six preparations of sample on a single batch sample by different analysts, the results of the ruggedness study are tabulated as below Table No.7. The % RSD values are less than 2.

Robustness

Robustness is the measure of the capacity of the analytical method to remain unaffected by small but deliberate variation in the procedure. The robustness of the method was evaluated by analysing the system suitability standard and evaluating system suitability parameter data after varying, individually, the HPLC pump flow rate (±0.2ml/min), column temperature (±5C) and detection wavelength (±2nm) shown in Table No.8.

Acceptance criteria

System suitability should pass as per test method at variable conditions.

Chromatographic conditions

Table No.1: HPLC method development parameters

S.No	HPLC method development parameters	
1	Column	C18, 250nm X 4.5µm, 5µ
2	Flow rate	0.5ml /min
3	Wavelength	226nm
4	Column temperature	40°C
5	Injection volume	20µL
6	Run time	10minutes
7	Diluents	Mobile phase
8	Elution	Isocratic

Table No.2: Specificity of bedaquiline

S.No	Name of the solution	Retention time in min
1	Blank	0
2	Bedaquiline (Standard)	2.9

Table No.3: Linearity of Bedaquiline

S.No	Concentration (µg/ml)	Peak area* (mv)
1	2	140449
2	4	280034
3	8	550730
4	16	1192850
5	32	2480424
6	64	5051223

*Average of six determinations

Table No.4: Results of Precision of bedaquiline

S.No	Intraday Names	Studies Peak area	Interday Names	Studies Peak area
1	Injection-1	560730	Injection-1	543987
2	Injection-2	569823	Injection-2	564298
3	Injection-3	573254	Injection-3	564123
4	Injection-4	563287	Injection-4	556359
5	Injection-5	554327	Injection-5	559836
6	Injection-6	567523	Injection-6	566281
7	AVG	564824	AVG	559147.3
8	STDEV	6226.954	STDEV	7530.611
9	%RSD	1.102	%RSD	1.34

Table No.5: Results of recovery of bedaquiline

S.No	Level of Addition %	Amount added (µg/ml)	Amount found	%Recovery ±Standard deviation*	%RSD
1	50	4	11.67 11.98 11.93	98.81±1.13	1.14
2	100	8	15.85 15.91 15.87	99.23±0.154	0.15
3	150	12	19.98 19.76 19.65	98.98±0.6859	0.692

*Average of three determinations

Table No.6: System suitability parameters

S.No	Parameters	Bedaquiline
1	Linearity	2-64 µg/ml
2	Regression equation	y = 79579x-55201
3	Correlation coefficient	R^2 = 0.9998
4	Retention time	2.9min
5	Run time	10min
6	Limit of detection (LOD)	0.29477 µg/ml
7	Limit of quantification (LOQ)	0.9825 µg/ml
8	Tailing factor	1.473
9	Theoretical Plate	2001

Table No.7: Results of Ruggedness of Bedaquiline

By changing the analysts

S.No	Analysts	Analyst 1	Analyst 2
1	Mean peak area	5140450	5132484
2	±Standard deviation*	7895.68	5528.22
3	%RSD	0.153	0.107

*Average of three determinations

By changing the instrument

S.No	Instrument	Instrument 1	Instrument 2
1	Mean peak area	5117639.6	5113809.3
2	±Standard deviation*	8443.41	4417.09
3	%RSD	0.164	0.086

*Average of three determinations

Table No.8: Robustness results for Bedaquiline

S.No	Parameters	Conditions	Tailing Factor	% RSD
1	Column Temperature	Decreased (-5°C)	1.10	0.18
		Increased (+ 5°C)	1.11	
2	Flow rate (ml/min)	Decreased (-2min/min)	1.365	0.40
		Increased (+2 min/min)	1.065	
3	Wavelength	Decreased(-2nm)	1.064	0.87
		Decreased(+2nm)	1.187	

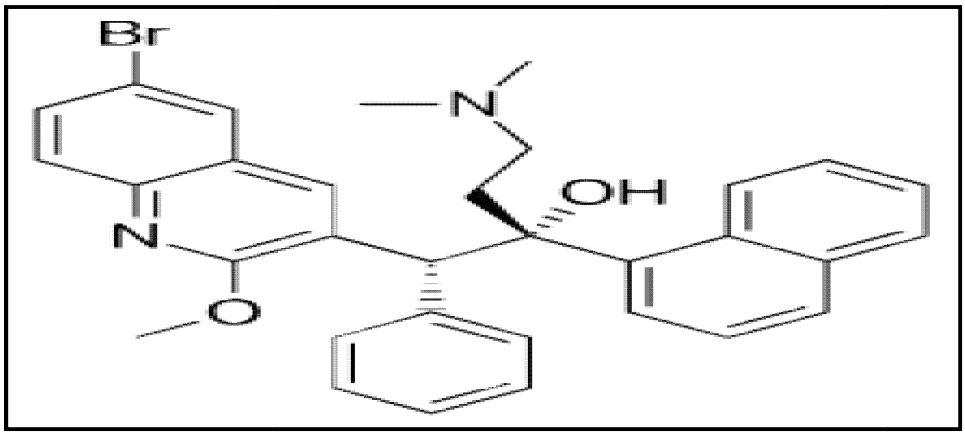

Figure No.1: Chemical structure of Bedaquiline

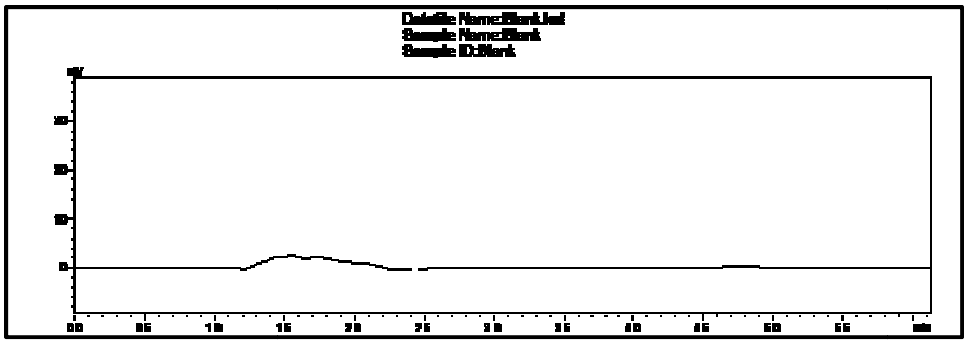

Figure No.2: Chromatogram of blank solution of bedaquiline

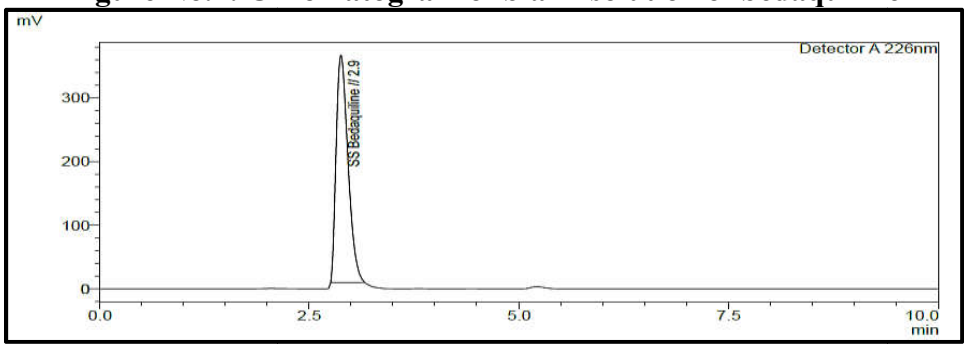

Figure No.3: Chromatogram of standard solution of Bedaquiline

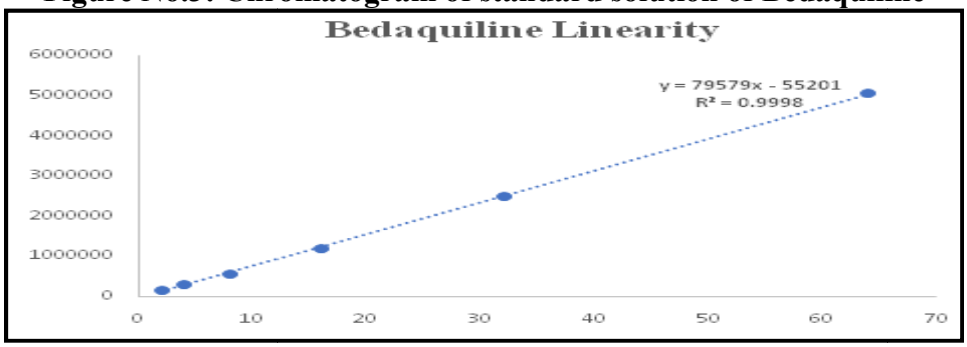

Figure No.4: linearity of Bedaquiline

CONCLUSION

The present analytical method was validated as per ICH guidelines and met the acceptance criteria. It was concluded that the developed analytical method was simple, accurate, economical and sensitive, and can be used for routine analysis of Bedaquiline in bulk drug and pharmaceutical dosage forms.

ACKNOWLEDGEMENT

We authors wish to thank 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.

BIBLIOGRAPHY

1. https://en.wikipedia.org/wiki/Bedaquiline.
2. Pooja B S, Shetty A S. Development and validation of UV spectrophotometric methods for the estimation of bedaquiline in bulk and pharmaceutical formulations, *World Journal of Pharmaceutical Research,* 7(7), 2018, 1579-1586.
3. Dousa M, Reitmajer J, Lustig P, Stefko M. Effect of chromatographic conditions on enantioseparation of Bedaquiline using polysaccharide-based chiral stationary phases in RP-HPLC, *Journal of Chromatographic Science,* 54(9), 2016, 1501-1507.

4. Pardhi V, Pant G, Flora S J. RP-HPLC method development and validation for Bedaquiline fumarate to evaluate its forced degradation behaviour and stability in official dissolution media, *Future J Pharma Sci,* 6(1), 2020, 1-10.
5. Momin M A, Rangnekar B, Das S C. Development and validation of a RP-HPLC method for simultaneous quantification of Bedaquiline (TMC207), moxifloxacin and pyrazinamide in a pharmaceutical powder formulation for inhalation, *J Liq Chrom and Related Tech,* 41(8), 2018, 415-421.
6. Hemanth Kumar K, Sudha V, Vijayakumar A, Padmapriyadarshini C. Simultaneous method for the estimation of Bedaquiline and Delamanid in human plasma using high performance liquid chromatography, *Int J Pharma and Pharma Sci,* 13(6), 2021, 36-40.
7. Metcalfe J, Gerona R, Wen A, Bacchetti P, Gandhi M. An LC-MS/MS-based method to analyze the anti-tuberculosis drug bedaquiline in hair, *The International Journal of Tuberculosis and Lung Disease: The Offial J Inter Union against Tuberculosis and Lung Disease,* 21(9), 2017, 1069-1070.
8. Alffenaar J W, Bolhuis M, van Hateren K, Sturkenboom M, Akkerman O, De Lange W, Greijdanus B, Van Der Werf T, Touw D. Determination of bedaquiline in human serum using liquid chromatography-tandem mass spectrometry, *Antimicrobial Agents and Chemotherapy,* 59(9), 2015, 5675-5680.
9. Vanavi P J, Rajput S J. Separation and characterization of novel degradation and process related impurities of bedaquiline bulk drug, *J Chrom Sci,* 2021, 1-14.
10. ICH, Q2A Text on Validation of Analytical Procedures, *U. S. Food and Drug Administration,* 1994.
11. ICH, Q2B Validation of Analytical Methodology, *U. S. Food and Drug Administration,* 1996.
12. ICH, Q2 (R1) Validation of Analytical Procedures: Text and methodology, *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use,* 2005, 1-17.

Please cite this article in press as: Chethan Gowda S *et al*. Validated RP-HPLC method for the estimation of Bedaquiline in bulk and tablet dosage form, *International Journal of Engineering and Robot Technology,* 8(2), 2021, 79-85.