ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Development and Validation of Simple UV Spectrophotometric Method for the Determination of Pretomanid

Vaishali Pardeshi*, Tushar Lokhande, Ashwini Shelke

Department of Pharmaceutical Chemistry, Mahatma Gandhi Vidyamandir's Pharmacy College, Nashik, Maharashtra, India

Article History:

Received on: 20 Sep 2022
Revised on: 14 Nov 2022
Accepted on: 17 Nov 2022

Keywords:

Pretomanid,
UV Spectrophotometric method,
Process validation,
ICH guidelines

ABSTRACT

In order to treat multidrug resistant TB, pretomanid, a nitroimidazooxazine antimycobacterial agent, is used with other antituberculosis medications. There is no technique for its analysis that uses spectroscopy, HPLC or HPTLC. Since a UV spectrophotometric approach for Pretomanid analysis must be developed. Utilizing a Shimadzu UV-2600, a quick, accurate, straightforward, and affordable UV spectrophotometric approach has been devised to determine Pretomanid. Solvent made of methanol to assess the bulk Pretomanid concentration. The detection process was placed at a wavelength of 321 nm. The parameters linearity, accuracy, precision, ruggedness, and robustness were taken into consideration during method validation in accordance with ICH Q2R1 criteria, as well as LOD and LOQ. It demonstrated linearity in the 10–30(g/mL) range at a predetermined max of 321 nm, and had a strong correlation coefficient (R2-0.997) and outstanding mean recovery (99.00–100.07%). In terms of intraday and interday precision, Pretomanid's% RSD was discovered to be 0.6366 and 0.666, respectively. Pretomanid identification using this approach was effective. The method's linearity, accuracy, repeatability, and reproducibility were statistically and empirically verified. The outcomes demonstrated the method's applicability for both routine examinations of protomanid bulks and industrial formulations. The suggested UV-Vis Spectrophotometric approach was verified in accordance with ICH requirements and found to be simple, accurate, precise and quick for the determination of Pretomanid.

*Corresponding Author

Name: Vaishali Pardeshi
Phone: 09156807993
Email: vaishali.pardeshi12@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v13i4.3939

Production and Hosted by

IJRPS | https://ijrps.com

INTRODUCTION

Pretomanid, a nitroimidazooxazine antimycobacterial agent, is used in conjunction with other antituberculosis medications to treat multidrug-resistant TB. [1] Pretomanid is (6S)-2-nitro-6-[[4-(trifluoromethoxy) phenyl] methoxy] in its chemical form. -6,7-dihydro-5H-imidazo[2,1-b] [2, 3] oxazine as shown in Figure 1 [2, 4]. In August 2019, the US Food and Drug Administration authorised Pretomanid. As part of a bedaquiline, pretomanid, and linezolid (BPaL) combination, this orally active medicine has been approved to treat people with pulmonary severe drug tuberculosis (TB) [3, 5]. Mycolic acid production is inhibited by pretomanid. This results in faulty cell wall construction, which eventually leads to bacterial cell death. It eradicates M. tuberculosis bacteria that are reproducing and those that are not. [6] For its analysis, no spectroscopic, HPLC, or HPTLC methods are avail-

able. Since there is a need to establish a Pretomanid analytical technique. This project's goal was to develop a precise, specific, repeatable, and accurate UV approach for the analysis of Pretomanid. The developed method was examined for linearity, accuracy, precision, robustness, ruggedness, LOD, and LOQ in accordance with ICH guidelines.

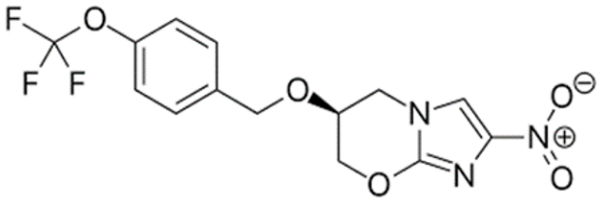

Figure 1: Pretomanid

It is branded as Pretomanid tablets 200mg and is available in tablet form. Methanol, DMSO (Dimethyl Sulfoxide), and ethanol are all solubalising solvents for it. Pretomanid is a prodrug that is activated by the Ddn nitroreductase enzyme in the body, resulting in a variety of active metabolites that are responsible for the drug's additional therapeutic effects, including nitric oxide induction. The reduced cofactor F420 is necessary for the pretomanid-activating nitroreductase enzyme, which is deazaflavin-dependent. The enzyme glucose-6-phosphate dehydrogenase reduces F420. [7] The pretomanid's imidazole ring is reduced at the C-3 position to produce the metabolites, one of which is a des-nitro derivative. Increased amounts of nitric oxide result from the synthesis of this derivative, which has bactericidal properties under anaerobic circumstances due to its function as a bacterial respiratory toxin. [8] Bactericidal activity against anaerobes has been linked to a reduction in antibiotic treatment duration. In a pharmacokinetic investigation of healthy people, the elimination half-life was shown to be 16.9-17.4 hours. A half-life of 18 hours is reported in an FDA briefing paper. [9]

MATERIALS AND METHODS

Pretomanid was received as a gift sample from Mylan, Hyderabad. Analytical grade Ethyl acetate, methanol and n-hexane were bought from Qualigen (India) Ltd., Mumbai, India.

All other chemicals come from S.D. Fine Chemical Ltd. in Worli, India, and are of analytical quality. And for all of the experimental work, volumetric glassware of class A grade was employed.

The UV spectrophotometric method was used on a SCHIMADZU-2600 Double Beam UV-Visible Spectrophotometer with two 10 mm matched quartz cells.

Solvent Selection

Various solvents such as methanol, ethanol, toluene, petroleum ether, acetonitrile, chloroform, n-hexane and acetone were tried for the estimation of Pretomanid. Methanol was selected as the solvent for the analysis of Pretomanid due to its easy availability, better solubility and cost-effectiveness,

Standard Stock Solution Preparation

Pretomanid was precisely weighed at 5 mg and then added to the 50 ml volumetric flask. Prepared up to 50 ml with the same substance after being dissolved in a minimum amount of methanol to achieve a concentration of 100 g/ml (100 ppm). After being diluted with methanol, 1 ml of the stock solution was added to a 10 ml volumetric flask. 10 g/ ml of the dilution was found to be present.

Determination of Maximum Absorption Wavelength

Using methanol as a blank, a 10 μg/ ml concentration solution was scanned between 200 and 400 nm, λmax was determined to be 321 nm from the UV spectra and was chosen as the analytical wavelength. Figure 2 depicted the UV spectrum of Pretomanid.

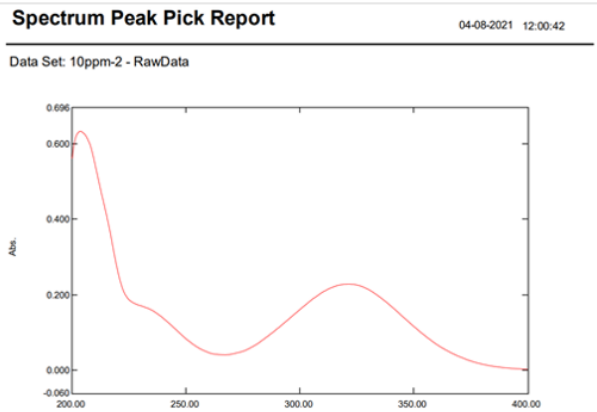

Figure 2: UV spectra showing Absorption Maxima (λ max) of Pretomanid

Preparation of Calibration Graph

In this procedure, a series of eight 10 ml volumetric flasks (10 - 24 μg/ml) were filled to capacity with methanol using aliquots of Pretomanid stock solution containing 100 μg /ml and ranging in size from 1.2 to 2.4 ml.

At 321 nm, the absorbance of various concentrations of liquids was measured in comparison to a blank. By graphing concentration vs absorbance, the calibration curve was created. At 321 nm, it was discovered that the sample was linear with a concentration range of 10 - 30μg/ ml.

Validation of Developed UV Spectrophotometric Method

Linearity

Between concentration and absorbance, a calibration graph was drawn. Pretomanid was linear at 321 nm, with a concentration range of 10 to 24 g/ml. The assay's limit of detection (LOD) and limit of quantitation (LOQ) were also determined [10].

Precision

The precision of the developed method was determined by intra-day and inter-day variation studies using only three concentrations of Pretomanid (10,14,18 ppm) 9 times. Intra-day studies and inter-day studies were determined by analyzing three sample solutions of concentrations 10,14,18. The mean, standard deviation and % RSD were determined [10]

Accuracy (Recovery Studies)

In this investigation, a blend of pure Pretomanid and common excipients was used. Calculations were made using the label claim and the typical final product weight. The admixture was diluted using the same method as before to achieve three concentrations: 80%, 100%, and 120% of the reference solution. By incorporating a known quantity of the sample solution into the standard stock solution, the investigation was conducted [10].

LOD and LOQ

The LOD and LOQ were estimated from formulas $3.3\sigma/S$ and $10\sigma/S$, respectively. Where, σ = the standard deviation of y-intercepts of regression lines S = the slope of the calibration curve [10].

Ruggedness

Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. The method's ruggedness was established by the determination of Pretomanid by change the analyst. The data were then subjected to statistical analysis and the results are expressed in mean, standard deviation and % RSD. [10]

Robustness

To determine the robustness of the method, absorbance was taken at wavelength change applied as ± 2 nm. The data were subjected to statistical analysis and the findings are shown as mean, standard deviation, and %RSD. [10]

RESULT AND DISCUSSION

Development and Validation of Method

Pretomanid is mainly insoluble in water but readily soluble in organic solvents like methanol and DMSO. Methanol utilised as the diluent resulted in a more favourable UV analysis result. The 321 nm maximum absorption wavelength (max) was predetermined.

Method Validation

Linearity

The calibration curve revealed linearity in the 10-24 g/ml (Table 1) range with a regression value of 0.997. The equation y = 0.0192+0.038 was obtained using linear regression of absorbance on concentration and has a correlation value (r) of 0.9974 (Figure 3). The maximum wavelength, or detecting wavelength, is 321 nm.

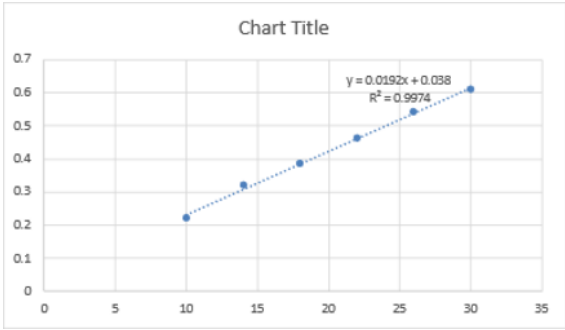

Figure 3: Calibration Curve of Pretomanid

Table 1: Calibration Parameters

Sr.No.	Concentration (μg/mL)	Absorbance
1.	10	0.220
2.	14	0.319
3.	18	0.384
4.	22	0.460
5.	26	0.540
6.	30	0.610

Accuracy (Recovery Studies)

The percent RSD and percentage recovery were computed. The mean percentage recovery and percent RSD were determined to be within acceptable ranges, both less than 2, indicating that the current study work is correct in its Pretomanid technique development. Calculations were made to determine the mean, standard deviation, and percentage relative standard deviation (%RSD). Table 2 presented the outcomes.

Precision

The assay's repeatability (intraday) and intermediate precision (inter-day) were quantified and expressed as a percentage of RSD. For this, the concentration solutions of 14 μg/mL, 18 μg/mL, and 22 μg/mL were tested three times a day, and the RSD%

Table 2: Recovery study

Drug	Initial amount Standard drug (100ppm) %	Amount added (200ppm)	Amount found	Recovery (%)	% RSD (n = 3)
Pretomanid	80%(10ppm)	8ppm	18.06	99.4	0.3
	100%(10ppm)	10ppm	19.9	99.7	0.4
	120%(10ppm)	12ppm	21.6	98.4	0.2

Table 3: Precision

Drug		Intra – Day			Inter – Day		
	Conc. (μg/ml)	Absorbance measured Mean ± SD %	RSD	Average Potency %	Mean ± SD	RSD	Average Potency %
Pretomanid	14	0.319± 0.004	0.14	104	0.315±0.002	0.7	103.4
	18	0.384± 0.004	1.07	100.1	0.394±0.003	0.6	100.6
	22	0.464± 0.003	0.7	100.8	0.474±0.003	0.7	102
		Mean RSD	0.63666			0.666	

Table 4: LOD and LOQ

Drug	LOD	LOQ
Pretomanid	0.037ppm	0.113ppm

Table 5: Ruggedness

Drug	Analyst-1			Analyst-2		
Concentration (ppm)	Mean Absorbance	% Amount found	% RSD (n=3)	Mean Absorbance	% Amount found	% RSD (n=3)
14	0.314	102	0.3	0.307	100	0.3
18	0.386	100.6	0.4	0.380	98.9	0.5
22	0.467	101.5	0.2	0.458	99.4	0.3

Table 6: Robustness data by taking absorbance at 321±2nm

Sr. No.	Concentration (ppm)	319nm	321nm	323nm	% Amount	% RSD
1.	14	0.315	0.315	0.314	102	0.15
2.	18	0.389	0.389	0.386	101.2	0.36
3.	22	0.466	0.467	0.467	101.4	0.10

was computed. (Table 3) In terms of intraday and interday precision, Pretomanid's% RSD was discovered to be 0.6366 and 0.666, respectively. Table 3 displayed the outcomes for intra-day and inter-day.

LOD and LOQ

Limit of Detection (LOD) and Limit of Quantitation (LOQ) estimates were used to determine the suggested method's sensitivity. The ICH indicates that LOD (DL, the detection limit) can be calculated as LOD = 3.3σ / S, and the limit of quantification (which they call QL, the quantitation limit) LOQ = 10σ / S. LOD and LOQ values for Pretomanid is given in Table 4. Here σ is the standard deviation of the response and S is the slope of the calibration curve. S is estimated from the slope of the calibration curve for the LOD and LOQ.

Ruggedness

Table 5 shows the ruggedness results

Robustness

The method's robustness is demonstrated by a slight planned change in the analytical wavelength. The

wavelength change applied as ±2 nm. The % RSD was found to be 0.15, 0.36, and 0.10 for variables shown in Table 6.

CONCLUSION

The suggested UV-Vis Spectrophotometric approach was found to be simple, precise, and accurate for the estimation of Pretomanid, and it was verified according to ICH requirements. As a result, this approach may be utilised for regular Pretomanid analysis without interfering with commonly used excipients in quality control laboratories, whether in bulk or in dosage formulations.

ACKNOWLEDGEMENT

The authors are thankful to Mylan Lab, Hyderabad, India for providing a gift sample. Also thankful to the Principal Mahatma Gandhi Vidyamandir's Pharmacy College Panchavati Nashik for providing facilities for carrying out this research work.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest.

REFERENCES

[1] A Bahuguna and D S Rawat. An overview of new antitubercular drugs, drug candidates, and their targets. *Medicinal research reviews*, 40(1):263–292, 2020.

[2] U Parveen, S Sultana, S F Heba, R Rafi, A Begum, and N Fatima. Pretomanid: A novel therapeutic paradigm for the treatment of drug-resistant tuberculosis. *Indian Journal of Tuberculosis*, 68(1):106–113, 2021.

[3] U.S. Food and Drug Administration. FDA briefing document: Briefing Document: Pretomanid Tablet, 200 mg, 2021. Meeting of the Antimicrobial Drugs Advisory Committee (AMDAC). June 06, 2019. Accessed on April 12, 2021.

[4] C K Stover, P Warrener, D R Vandevanter, D R Sherman, T M Arain, M H Langhorne, and W R Baker. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. *Nature*, 405(6789):962–966, 2000.

[5] AMDAC. FDA Briefing Document Pretomanid Tablet, 200 mg Meeting of the Antimicrobial Drugs Advisory Committee (AMDAC), 2019. Accessed on June 06, 2019.

[6] S Tyagi, E Nuermberger, T Yoshimatsu, K Williams, I Rosenthal, N Lounis, and J Grosset. Bactericidal activity of the nitroimidazopyran PA-824 in a murine model of tuberculosis. *Antimicrobial agents and chemotherapy*, 49(6):2289–2293, 2005.

[7] A J Lenaerts, V Gruppo, K S Marietta, C M Johnson, D K Driscoll, N M Tompkins, and I M Orme. Preclinical testing of the nitroimidazopyran PA-824 for activity against Mycobacterium tuberculosis in a series of in vitro and in vivo models. *Antimicrobial agents and chemotherapy*, 49(6):2294–2301, 2005.

[8] U Manjunatha, H I Boshoff, and C E Barry. The mechanism of action of PA-824: novel insights from transcriptional profiling. *Communicative & integrative biology*, 2(3):215–218, 2009.

[9] R Singh, U Manjunatha, H I Boshoff, Y H Ha, P Niyomrattanakit, R Ledwidge, and C E Barry 3rd. PA-824 kills nonreplicating Mycobacterium tuberculosis by intracellular NO release. *Science*, 322(5906):1392–1395, 2008.

[10] ICH Harmonised Tripartite Guideline. Validation of analytical procedures: text and methodology, in International Conference on Harmonization (ICH), Q2(R1), 2005. IFPMA, Geneva, Switzerland.