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DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR ESTIMATION OF DELAMANID IN TABLET DOSAGE FORM

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ABSTRACT

Objective: To develop and validate simple, rapid, linear, accurate, precise and economical UV Spectroscopic method for estimation of Delamanid in tablet dosage form. **Methods:** The drug is soluble in Methanol. The drug was identified in terms of solubility studies and on the basis of melting point done on melting point apparatus of Equiptronics. It showed absorption maxima were determined in Methanol. The drug obeyed the Beer's law and showed good correlation of concentration with absorption which reflect in linearity. The UV spectroscopic method was developed for estimation of Delamanid in tablet dosage form and also validated as per ICH guidelines. **Results:** The drug is freely soluble in Methanol, soluble in ethanol and insoluble in water. So, the Methanol is used as a diluent in method. The melting point of Delamanid was found to be 189-190˚C (uncorrected). It showed absorption maxima 320 nm in Methanol. On the basis of absorption spectrum the working concentration was set on 15µg/ml (PPM). The linearity was observed between 5-25 µg/ml (PPM). The results of analysis were validated by recovery studies. The recovery was found to be 98.8, 98.0 and 98.3% for three levels respectively. The % RSD for precision and ruggedness was found to be 0.72% and 0.52% respectively. **Conclusion:** A simple, rapid, linear, accurate, precise and economical UV Spectroscopic method has been developed for estimation of Delamanid in tablet dosage form. The method could be considered for the determination of Delamanid in quality control laboratories.

KEYWORDS: Delamanid, UV Spectrophotometer, Melting Point, Assay Method, Validation, Accuracy, Linearity, Ruggedness, Precision.

INTRODUCTION

Delamanid is an anti-tubercular drug, utilized in combination with bedaquiline for treating active multidrug-resistant tuberculosis in adults when other treatments prove ineffective or elicit no response. The chemical structure of Delamanid comprises a nitro-imidazole core attached to a lateral tail formed of aromatic and aliphatic rings connected by an oxygen atom, rendering a stretched form to the overall structure.[1,2] It is the initial medication in a novel family of anti-TB medications called nitroimidazoles. IUPAC name of delamanid is (2R)-2-methyl-6-nitro-2-[[4-[4-[4-(trifluoromethoxy)phenoxy]piperidin-1-yl] phenoxy] methyl]-3H-imidazo[2,1-b][1,3]oxazole.[3,4] A medicine called delamanid is used to treat people with lung-related multidrug-resistant tuberculosis. Delamanid is a prodrug that requires biotransformation via via the mycobacterial F420 coenzyme system, including the deazaflavin dependent nitroreductase (Rv3547), to mediate its antimycobacterial activity 2 against both growing and nongrowing mycobacteria.[5] Upon activation, the radical intermediate formed between delamanid and desnitro-imidazooxazole derivative 5 is thought to mediate antimycobacterial actions via inhibition of methoxy-mycolic and keto-mycolic acid synthesis, leading to depletion of mycobacterial cell wall components and destruction of the mycobacteria.[6]

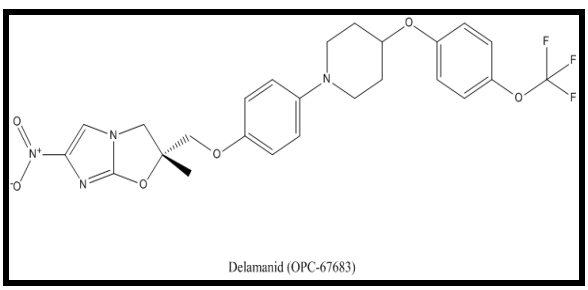

Fig. 1: Chemical Structure of Delamanid.

From literature review it's found that one method was reported on Simultaneous quantitation of delamanid (OPC-67683) and its eight metabolites in human plasma using UHPLC-MS/MS[7], also plasma method reported on liquid chromatography-Tandem mass spectrometry methods for determination of delamanid in mouse plasma and lung.[8] But no method was reported on estimation of Delamanid in tablet dosage form for UV spectroscopic method. This indicates that so far no UV method exists for the estimation and determination of Delamanid in tablet dosage forms.

MATERIALS AND METHODS

- **Instruments**
  Shimadzu double beam UV-visible spectrophotometer 1700 Ultra with matched pair Quartz cells corresponding to 1 cm path length and spectral bandwidth of 1 nm, Bath sonicator and citizen weighing balance. Melting point apparatus of Equiptronics were used.
- **Materials**
  Delamanid was obtained as a gift sample. Delamanid tablets were procured from local pharmacy. Methanol was used throughout the experiment as a diluent. Freshly prepared solutions were employed.

Method development

A. Determination of ƛ max (10 PPM)[9, 10]

50 mg weighed amount of Delamanid was dissolved into 100 ml of volumetric flask with Methanol. Pipette out 1 ml and added in 50 ml of volumetric flask dissolved and diluted up to the mark with Methanol. This solution was subjected to scanning between 200-400 nm and absorption maximum was determined.

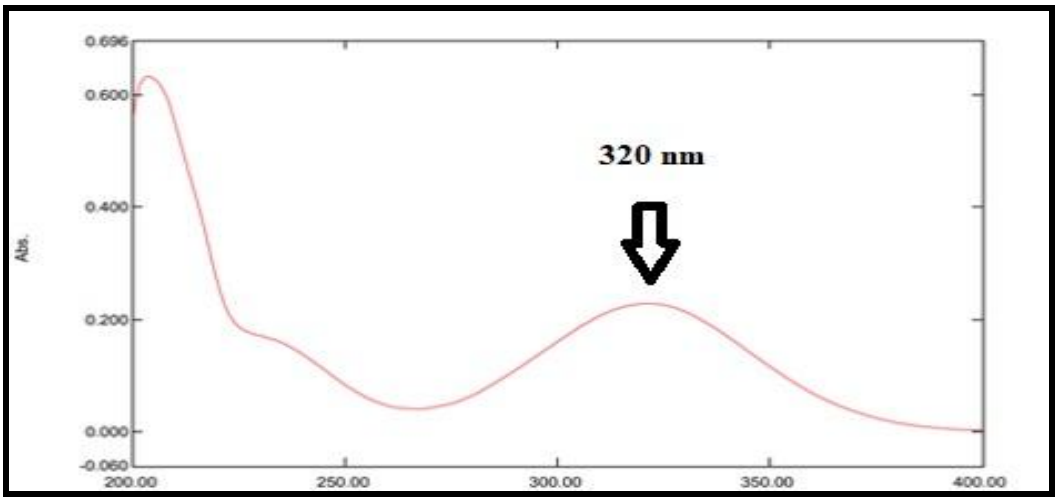

Fig. 2: Calibration Curve

B. Preparation of Working concentration[9]

Preparation of Standard stock solution

Standard stock was prepared by dissolving 50 mg of Delamanid in 100 ml of Methanol to get concentration of 500µg/ml (PPM).

Preparation of Standard solution

Pipette out 1.5 ml from standard stock solution and diluted up to 50 ml with Methanol to get concentration of 15µg/ml (PPM).

C. Procedure for UV reading

Blank Solution: (For Auto zero)

Fill the cuvette with Methanol. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Standard Solution

Fill the cuvette with standard solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

Sample Solution

Fill the cuvette with sample solution. Wipe it with tissue paper properly then placed inside the chamber. Note down the reading.

D. Procedure for sample preparations[10]

For analysis of commercial formulations; twenty tablets are taken weighed it and powdered. The powder equivalent to 50 mg of Delamanid was accurately weighed and transferred into the 100 ml of volumetric flask, added 60 ml Methanol, the solution was sonicated for 20 min. After sonication cool the flask and diluted upto 100 ml with Methanol. Filtered the solution through whatmann filter paper. Pipette out 1.5 ml of the above solution and diluted up to 50 ml with Methanol. The absorbance was measured at 320 nm. The absorbance was recorded:

Table 1: Absorbance of Dosage Form.

Otsuka Pharmaceutical Limited Deltyba® 50 (50 mg)		
Sr. No.	**Sample**	**Absorbance**
1	Blank	0.0001
2	Standard	0.5598
3	Sample	0.5536

Table 2: Dosage Form Specifications.

Type	Company	M.D.	E.D.	Batch No.	Average weight (g)	Assay (%)
1	Otsuka Pharma LTD Deltyba® 50	08/2024	05/2026	ZDS 548	0.214	98.89

E. Method of validation[11-14]

The proposed method was developed by using linearity, accuracy, precision and ruggedness as per ICH guidelines, 1996.

Linearity

The linearity of the proposed assay was studied in the concentration range 5 - 25 PPM at 320nm. The calibration data showed a linear relationship between concentrations.

Table 3: Linearity Studies.

Sr. No.	Sample Concentration	Absorbance
1	5 PPM	0.1735
2	10 PPM	0.3693
3	15 PPM	0.5597
4	20 PPM	0.7798
5	25 PPM	0.9952
Correlation coefficient		**0.999**

Accuracy

To ensure the accuracy of the method, recovery study was performed by preparing 3 sample solutions of 80, 100 and 120% of working concentration and adding a known amount of active drug to each sample solution and dissolved in 100ml of volumetric flask with Methanol and measuring the absorbance at 320nm.

Table 4: Accuracy Studies.

SPECTROPHOTOMETRIC METHOD			
Accuracy (%)	**Qty weighed (mg)**	**Qty found (mg)**	**Recovery (98-102%)**
80	0.8	0.79	98.75 ~ 98.8
100	1	0.98	98.0
120	1.2	1.18	98.33 ~ 98.3

Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies. Five sample solutions were made and the % RSD was calculated.

Table 5: Precision studies.

Sr. No.	Sample Solution	Absorbance
1	Sample Solution 1	0.5596
2	Sample Solution 2	0.5526
3	Sample Solution 3	0.5532
4	Sample Solution 4	0.5485
5	Sample Solution 5	0.5536
MEAN		**0.5535**
SD		**0.0040**
% RSD		**0.72**

Ruggedness

Ruggedness is a measure of the reproducibility of a test result under normal, expected operating condition from instrument to instrument and from analyst to analyst.

Table 6: Results for Ruggedness Studies.

Sr. No.	Analyst	Results	Mean	% Assay	% RSD
1	Analyst 1	0.5533	0.5539	98.94	0.5127 ~ 0.52
		0.5545			
2	Analyst 2	0.5560	0.5579	99.66	
		0.5598			

RESULTS

1. Solubility of Delamanid

Solubility test was passed as per criteria.

Table 7: Results for solubility studies

Sr. No.	Title	Result
1	Methanol, DMSO	Freely Soluble
2	Ethanol	Soluble
3	Water	Insoluble

2. Melting point of Delamanid

The melting point of Delamanid was found to be 189-190˚C (uncorrected).

3. Results for linearity for assay method of Delamanid [Conc. Vs Absorbance]

The linearity of method was determined at concentration level ranging from 5 to 25 µg/ml (PPM). The correlation coefficient value was found to be **(R^2) 0.999.**

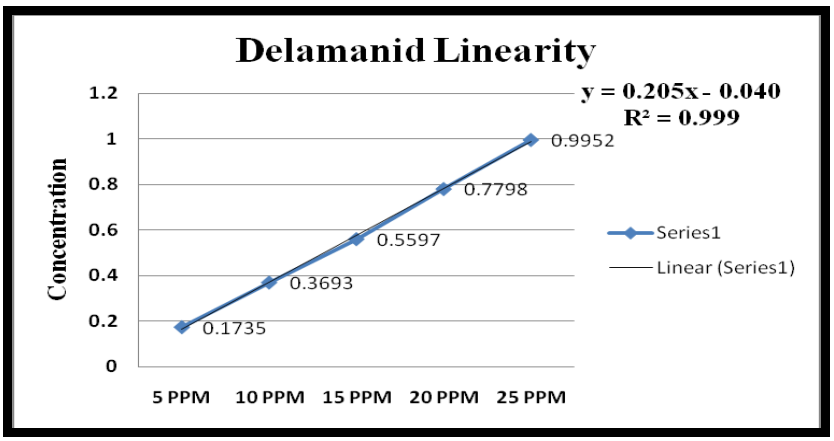

Fig. 3: Delamanid Standard Curve.

4. Results for accuracy for assay method of Delamanid

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out and the percentage recovery were calculated and represented in Table - 4. The high percentage of recovery indicates that the proposed method is highly accurate. Accuracy results were found within acceptance criteria that are within 98-102%.

5. Results for precision for assay method of Delamanid

The % RSD for different sample of precision was found to be 0.72 and it is within acceptance criteria represented in Table - 5.

6. Results for ruggedness for assay method of Delamanid

The % RSD for different sample of ruggedness was found to be 0.52 and it is within acceptance criteria represented in Table - 6.

CONCLUSION

A method for the estimation of Delamanid in tablet form has been developed. From the spectrum of Delamanid, it was found that the maximum absorbance was 320 nm in Methanol. A good linear relationship was observed in the concentration range of 5-25 µg/ml (PPM). The high percentage recovery indicates high accuracy of the method. This demonstrates that the developed spectroscopic method is simple, linear, accurate, rugged and precise for the estimation of Delamanid in solid dosage forms. Hence, the method could be considered for the determination of Delamanid in quality control laboratories.

ABBREVARTIONS

1. PPM - Parts per Million
2. nm - Nanometer
3. TB - Tuberculosis
4. HPLC - High Performance Liquid Chromatography
5. UV - Ultra violet
6. FDA - Food and Drug Administration

7. NaOH - Sodium Hydroxide
8. ICH - International Council for Harmonization
9. RSD - Relative Standard Deviation
10. SD - Standard Deviation
11. Qty - Quantity
12. C - Celsius
13. M.D. - Manufacturing Date
14. E.D. - Expiry Date

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