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Development and Validation of a simple UV spectrophotometric method for the determination of Delamanid

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ABSTRACT

Using a Shimadzu UV-2600, a quick, precise, easy, and affordable UV spectrophotometric approach has been created. Solvent made with methanol to assess the bulk Delamanid content. A wavelength of 320 nm was used for the detection process. The parameters linearity, accuracy, precision, ruggedness, robustness, LOD, and LOQ were taken into consideration during method validation in accordance with ICH Q2R1 criteria. It demonstrated linearity in the range of 60-360 (/mL) at a predetermined λmax of 320 nm, and it had a strong correlation coefficient (R2-0.996) and outstanding mean recovery (99.00-100.07%). Determination of Delamanid used this technique effectively. The method's linearity, accuracy, repeatability, and reproducibility were statistically and by recovery experiments confirmed. The outcomes demonstrated the method's applicability for both regular Delamanid bulk analysis and commercial formulations.

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INTRODUCTION

The drug delamanid is efficient in treating MDR TB. It is referred to by its trademark, Deltyba. 1. It is the first of a brand-new class of anti-TB drugs known as nitroimidazoles [1]. The chemical name for delamanid is (2R)-2-[(4-(trifluoromethoxy)phenoxy]. -1-piperidinyl]] -6-nitro -2-[(4-4-[4-(trifluoromethoxy)phenoxy]methyl]. Dihydroimidazo[2,1-b] [2, 3] oxazole as shown in Figure 1. Delamanid is a medication used to treat individuals with multidrug-resistant tuberculosis (TB) that affects the lungs. By preventing the production of mycobacterial cell wall constituents such as methoxy mycolic acid and ketomycolic acid, it functions as a prodrug [1, 2]. For delamanid's analysis, no spectroscopic, HPLC, or HPTLC approach is available. thus Delamanid's analytical procedure needs to be developed. The current study's objective was to create a precise, repeatable, accurate UV approach for the analysis of delamanid. The. The developed method looked for linearity, accuracy, precision, robustness, ruggedness, LOD, and LOQ in accordance with ICH guidelines [4].

It is branded as Deltyba 50mg available in tablet form. Methanol, DMSO (Dimethyl Sulfoxide), and DMF are all solubilizing solvents for it. Keto mycolic acid and methoxy mycolic acid, two crucial mycolic acid constituents, have been demonstrated to be prevented by delamanid. Delamanid's inhibitory effect is restricted to mycobacteria since mycolic acids are only present in the cell walls of mycobacteria and are not present in the cell walls of other Gram positive or Gram-negative bacteria [3, 5]. Breaking the cell wall enables increased medication penetration and hence a shorter treatment schedule since these acids make it harder for pharmaceuticals to

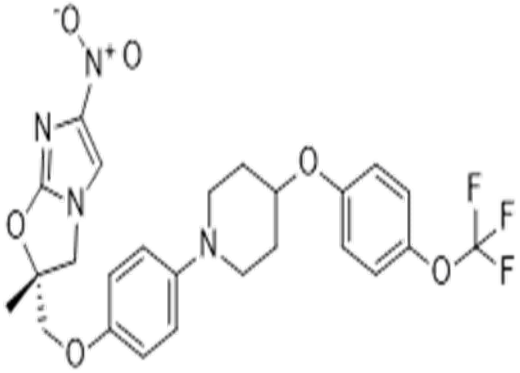

Figure 1: Delamanid

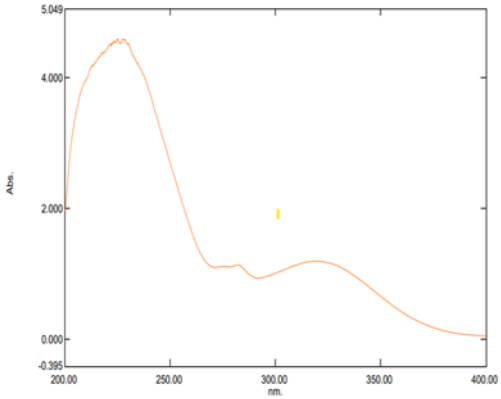

Figure 2: UV spectra showing Absorption Maxima (λ max) of Delamanid

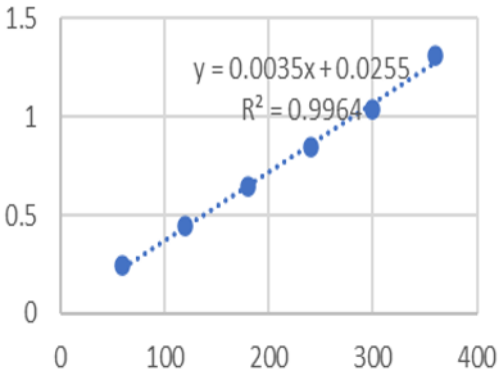

Figure 3: Calibration Curve of Delamanid

permeate the mycobacterium's cell wall [6, 7].

MATERIALS AND METHODS

Delamanid pure drug was received as a gift sample from Mylan, Hyderabad. Analytical grade Ethyl acetate, methanol and n-hexane were acquired 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. On a SCHIMADZU-2600 Double Beam UV-Visible Spectrophotometer with two 10 mm matched quartz cells, the UV spectrophotometric technique was used.

Solvents such as methanol, ethanol, toluene, petroleum ether, acetonitrile, chloroform, n-hexane and acetone were tried for the estimation of Delamanid. Because of easy availability, better solubility and cost effectiveness. Methanol was chosen as the solvent for the Delamanid analysis.

Table 1: Calibration Parameters

Sl.No.	Concentration (μg/mL)	Absorbance
1	60	0.247
2	120	0.447
3	180	0.646
4	240	0.845
5	300	1.038
6	360	1.314

Standard Stock Solution Preparation

Delamanid was carefully measured at 50 mg and added to the volumetric flask at 50 ml. Prepared up to 50 ml with the same after being dissolved in a small amount of methanol to obtain a concentration of 1000 /ml(1000 ppm). 3.6 ml of the stock solution was transferred into a 10 ml volumetric flask and diluted with methanol. The dilution was observed to contain 360μg/ ml.

Determination of Wavelength of Maximum Absorption

Using methanol as a blank, the 360 g/ ml concentration solution was scanned between 200 and 400 nm. (Figure 2) λmax was determined to be 320 nm from the UV spectra and was chosen as the analytical wavelength. The UV spectrum of Delamanid was shown in Figure 2.

Preparation of Calibration Graph

In this method, 0.6-3.6 ml of the aliquots of stock solution of Delamanid containing 1000μg/ ml were transferred into six 10 ml volumetric flasks in a sequence. (60 - 360 μg/ml) and made up to the volume with methanol.

At 320 nm, the absorbance of solutions of various concentrations was measured in comparison to a blank. By graphing concentration vs absorbance, the calibration curve was created.

At 320 nm, it was discovered that the sample was linear with a concentration range of 60 to 360 μg / ml.

Table 2: Recovery study

Drug	Initial amount Standard drug(100ppm) %	Amount added (200ppm)	Amount found	Recovery (%)	% RSD (n = 3)
Delamanid	80%(10ppm)	180ppm	181.2	101.2	0.07
	100%(10ppm)	200ppm	197.3	98.9	0.2
	120%(10ppm)	220ppm	218.6	99.3	0.3

Table 3: Precision

Drug		Intra – Day			Inter – Day		
Delamanid	Con. (μg/ml)	Absorbance measured Mean $\pm$ SD %	RSD	Average Potency %	Mean $\pm$ SD	RSD	Average Potency %
120	0.455$\pm$ 0.005	1.1	104	0.462$\pm$0.002	0.6	103.4	
180	0.648$\pm$ 0.006	1.1	100.1	0.66$\pm$0.003	0.5	100.7	
240	0.842$\pm$ 0.004	0.45	100.8	0.851$\pm$0.006	0.14	98.2	
	Mean RSD	0.8			0.4		

Validation of Developed UV Spectrophotometric Method

Linearity

A calibration graph was created between concentration and absorbance. With a concentration range of 60–360 μg / ml at 320 nm, delamanid was linear. Additionally, the assay's limit of quantitation (LOQ) and limit of detection (LOD) were determined.

Precision

By conducting intra-day and inter-day variation trials using just three concentrations of Delamanid (120, 180, and 240 ppm) nine times, the suggested method's accuracy was evaluated. Intra-day studies and inter-day studies were determined by analysing three sample solutions of concentrations 120,180,240. The mean, standard deviation and % RSD were calculated.

Accuracy (Recovery Studies)

A blend of pure Delamanid and common excipients were used in this investigation. 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 carried out.

LOD and LOQ

The set of six calibration curves used to assess the method's linearity was utilised to estimate LOD=3.3/S and LOQ=10/S Where σ is the standard deviation of the regression line's y-intercepts, The calibration curve's slope is denoted by S.

Ruggedness

The degree of repeatability of outcomes under various settings is known as ruggedness. Delamanid's decision to switch the analyst proved the robustness of the process.

The method's ruggedness was found by changing the analyst. The findings of the statistical analysis of the data are given as mean, standard deviation, and percent RSD.

Table 4: LOD and LOQ

Drug	LOD	LOQ
Delamanid	22.9ppm	69.54ppm

Robustness

For determi ation of the robustness of the method, wavelength change was applied as $\pm$2 nm.

Following the statistical analysis of the data, the results are presented as mean, standard deviation, and % RSD.

Table 5: Ruggedness

Drug Concentration (ppm)	Analyst-1			Analyst-2		
	Mean Absorbance	%Amount found	% RSD (n=3)	Mean Absorbance	%Amount found	% RSD (n=3)
120	0.447	102	0.8	0.440	98.7	0.3
180	0.646	100.6	0.4	0.651	97.7	0.5
240	0.845	101.5	0.7	0.858	99.10	0.3

Table 6: Robustness data of UV-Vis spectrophotometric method by taking absorbance at 320±2nm

Sl. No	Concentration (ppm)	318nm	320nm	322nm	%Amount	%RSD
1	120	0.436	0.438	0.437	97.9	0.2
2	180	0.660	0.661	0.665	101	0.3
3	240	0.868	0.860	0.865	99.9	0.4

RESULTS AND DISCUSSION

Method Development and Validation

Delamanid is easily soluble in organic solvents including methanol, DMF, and DMSO but practically insoluble in aqueous media. A few millilitres of methanol used as the diluent during the development phase produced a more favourable UV analysis result. The predetermined maximum absorption wavelength (λmax) was 320 nm.

Method Validation

Linearity

It was discovered that the calibration curve exhibits linearity in the range of 60-360μg/ml with a regression coefficient of 0.996 (Figure 3). With a correlation coefficient (r) of 0, the equation y = 0.0035+0.0255 was produced via linear regression of absorbance on concentration. 996. The maximal wavelength at 320 nm is visible for the detecting wavelength.

Accuracy (Recovery Studies)

RSD and the percentage recovery were computed. The present study work is correct in the technique creation of Delamanid since the mean percentage recovery and RSD were discovered to be within limits and less than 2. Calculations were made to determine the mean, standard deviation, and percentage relative standard deviation (%RSD). Table 2 presented the outcomes.

Precision

The repeatability (intraday) and intermediate precision (inter-day) of the assay were used to calculate its precision, which was reported as RSD%. For this,120 μg/mL, 180 μg/mL and 240μg/mL concentration solution were measured three times a day and RSD% was calculated. (Table 3). In terms of intra- and inter-day precision, Delamanid's% RSD was discovered to be 0.6366 and 0.666, respectively. Table 3 displayed the results for intra-day and inter-day. The devised technique's intra-day and inter-day precision study (Table 1), where all the RSDs were 2%, demonstrated appropriate sample stability and method dependability.

LOD and LOQ

Limit of Detection (LOD) and Limit of Quantitation (LOQ) estimates were used to determine the suggested method's sensitivity. Results are shown in Table 4.

Ruggedness

Table 5 displays the results for toughness. The method's robustness is demonstrated by the low percentage RSD value.

Robustness

A slight planned modification in the analytical wavelength proved the method's robustness. The wavelength change applied as ±2 nm. At the selected variable wavelength, the amount of formulation was found. The % RSD was found to be 0.15, 0.36,0.10 for variables shown in Table 6.

CONCLUSION

The proposed UV-Vis Spectrophotometric approach was validated in accordance with ICH requirements and proved to be quick and accurate for estimating Delamanid. Therefore, without interfering with routinely used excipients, this method can be quickly and conveniently employed for routine analysis of Delamanid in quality control laboratories, whether in bulk or dosage formulations.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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