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Research Article

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Development and Validation of RP-HPLC Method for Estimation of Delamanid

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Abstract Reverse-phase high-performance liquid chromatography was developed and validated as a simple, accurate, precise and sensitive method for estimation of Delamanid in bulk and pharmaceutical dosage form. Chromatography was carried out on C18 column using a mixture of HPLC grade Methanol; 0.1% ortho phosphoric acid (81/19) as the mobile phase at a flow rate of 1.1 mL/min. The detection was carried out at 222 nm. It was determined that the retention time was 4.278 ± 0.12 min. The method produces linear response in the concentration range of 10 to 50 µg/mL of Delamanid. The method precision for the determination of assay was below 2%RSD. Accuracy was found in the limit. The method is useful in the quality control of bulk and pharmaceutical dosage form.

Keywords: Dosage forms, RP-HPLC, Delamanid, and method validation

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M. tuberculosis* or MTB), remains the leading cause of death from an infectious disease globally, currently outranking HIV [1]. In 2016 alone, 10.4 million became ill with TB, including 490,000 people who developed or acquired multidrug-resistant TB (MDR-TB), against which the two most potent first-line drugs, isoniazid and rifampicin, are ineffective. In terms of mortality, there were 1.3 million deaths from TB in people without HIV infection, and an additional 374,000 deaths in people with HIV [1]. Despite high cure rates for drug susceptible (DS) TB, the latest data for drug resistant TB shows that outcomes remain poor with treatment success reported for 54% and 30% of MDR-TB and XDR-TB (extensively drug-resistant TB; MDR-TB plus additional resistance to a fluoroquinolone and a second-line injectable agent (amikacin, capreomycin or kanamycin)), respectively [1].

Drug-resistance undermines TB-control efforts, and poses the threat of TB disease becoming programmatically incurable as bacteria progressively acquire resistance to additional drugs. However, treatment of drug-resistant TB requires the use of a combination of older second-line TB medicines, including painful injectable agents (i.e. aminoglycosides and cyclic peptides), that have often undergone only limited testing for the treatment of TB, and are known to cause severe side effects including irreversible ototoxicity, hyperpigmentation, neuropsychiatric side-effects, hepatotoxicity, and bone marrow toxicity [2,3].

For decades, the pipeline for tuberculosis research and development has been largely empty [4]. Otsuka Pharmaceutical Co., Ltd, founded in Japan in 1964, decided to focus on TB as one of its first research priorities in the early 1990s, and thus committed itself to finding new treatment options for TB when most companies were closing

their TB programs [5]. The discovery pathway for the treatment of TB is much more difficult than for general antibiotics. First, TB bacilli replicate slowly, thus requiring a longer time for cultures to grow and disease to develop in animal models in order to allow a compound's activities to be assessed. Second, as a biosafety level 3 pathogen, MTB requires facilities that must meet stringent biosafety requirements for both in vitro and in vivo work. Third, the unique features of pulmonary TB lesions such as hypoxia and/or cavitation may reduce intrinsic drug susceptibility, resulting in very limited available candidate seed or lead structures [6]. Finally, and perhaps most challenging, is the fact that human TB pathology manifests as discrete microenvironments within which the bacterium resides, which few animal models can faithfully mimic; thus, the ability of preclinical data to predict success in humans is limited [6].

After years of persistent research with various compounds failing to show efficacy in animal studies, in-house Otsuka scientists screening tens of thousands of compounds identified OPC-67683 (later named delamanid) (Fig. 1). Based on its favourable preclinical features, it was chosen to go into clinical trials to evaluate its pharmacokinetics (PK), safety and efficacy. After results from a phase 2b trial showing its efficacy for the treatment of pulmonary MDR-TB, delamanid received regulatory approval for treatment of adult MDR-TB patients in April 2014 from the European Medicines Agency (EMA) and is one of only two anti-TB drugs in new classes successfully developed in the last 50 years. This review describes the discovery effort, preclinical testing, the microbiological profile, and results from clinical evaluations of delamanid.

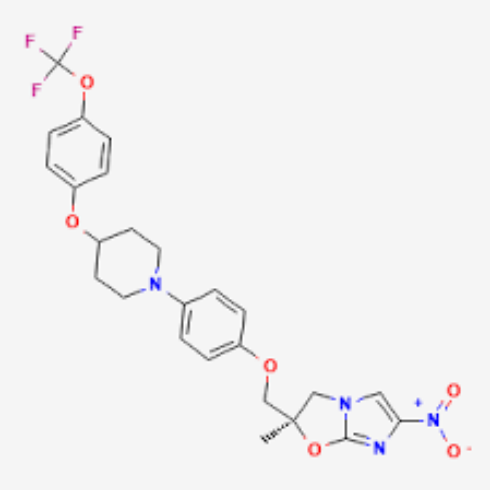

Figure 1: Chemical structure of Delamanid

Methods

Chemical and reagents

Delamanid, Methanol, 0.1%Ortho Phosphoric Acid, HPLC

Chromatographic conditions

A prominence isocratic HPLC system (Shimadzu LC-2030Plus High Performance liquid chromatography with auto sampler Kromasil UV detector) column symmetry C18 (300×3.9mm,5µm). A 20µL injection syringe was used for sample injection. HPLC grade Methanol and 0.1% Orthophosphoric acid in the ratio of (81:19 v/v) were used for the preparing the mobile phase. A freshly prepared Methanol and 0.1% Orthophosphoric acid in the ratio of (81:19 v/v) was used as the mobile phase. The solvents was filtered through a 0.45µ membrane filter and sonicated before use. The flow rate of the mobile phase was maintained at 1.1mL/min., column temperature was maintained at room temperature and detection was performed at 222 nm using a UV detector.

Preparation of mobile phase

Mix a HPLC grade Methanol and 0.1% Ortho Phosphoric acid in the ratio of (81:19 v/v) degas in ultrasonic water bath for 5 minutes. Filter through 0.45µ filter under vacuum filtration.

Diluent preparation
Mobile phase as diluents.

Standard solution preparation:
Accurately weigh and transfer 100mg of Delamanid working standard in to 100mL volumetric flask add about 40 mL HPLC grade water and sonicated to dissolve it completely and the make the same solvent at the appropriate volume. (Stock solutions). Mix well and filter through 0.45 µm filter. (1000 µg/ml or PPM) Further 10 ml of stock solution transferred into 100 ml volumetric flask and diluted up to the mark with diluent and mixed well. (Concentration of Standard Solution: 100 µg/ml or PPM).

Sample solution preparation:
Weight 20 tables and calculate the average weight Triturate 20 tablets to a fine powder. Weighed accurately equivalent to 100mg of Delamanid, transfer into a 100ml volumetric flask, add 40ml of HPLC grade water, shake for 5min and sonicated for 30min and make up the volume with HPLC grade water, and filter through 0.45micron membrane filter. Pipetted out 10 ml of filtrate, transfer in 100 ml volumetric flask and make up the volume with HPLC grade water.

Method Validation
Specificity:
Blank, API and Drug product were injected. Chromatographs were observed.
Linearity:
The linearity of the method was demonstrated over the concentration range of 10-50 µg/ml of the target concentration. Aliquots of 10, 20, 30,40 and 50 µg /ml were prepared from above stock solution. Different concentrations of the pure drug were injected into the chromatographic system. Each concentration was evaluated five times and the corresponding mean peak area ratios (response factor) were plotted as a function of concentrations.

Precision Method
Studies examining variations within and between days proved how accurate the system was. In the intra-day and Interday studies, three repeated injections of 3 different concentrations (10, 30 and 50ug/ml) of standard solution was made and the response factor of drug peak and % RSD were calculated.
Accuracy
A study recovery of Delamanid from spiked placebo was conducted at three different spike levels i.e.80%, 100% and 120% Samples were prepared with Delamanid active pharmaceutical ingredients equivalent to about the target initial concentration of Delamanid. By the suggested technique, sample solutions were made in triplicate for every spike level.

LOD and LOQ:
According to ICH, Limit of Detection (LOD), and limit of quantification (LOQ) were calculated using the response's standard deviation and slope. Equation (1) was used to determine LOD, and Equation (2) was used to determine LOQ (ICH harmonized tripartite guideline, 2005)
Equation (1)–LOD=3.3σ/s Equation (2) LOQ =10σ/s
where σ corresponds to standard deviation of the y-intercepts of regression line and S is the slope of calibration curve.

System suitability
System suitability tests ensure that the chromatography system operates at a suitable level and are an important part of method development. Retention time (Rt), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 30µg/ml.

Results

Development and Optimization of the HPLC Method

For the HPLC method to generate a suitable eluted component separation, the HPLC conditions were optimized. Satisfactory chromatographic separation was achieved with Kromasil100-5-C18 column (300×3.9 mm, 5 µm) column. Optimized mobile phase consisted of a mixture of composed of HPLC grade Methanol; 0.1% ortho phosphoric acid (81/19v/v). Above mobile phase ratio provided good resolution of Delamanid. Flow rate of the mobile phase was set at 1.1 mL/min and injection volume 20 ml. Analytes were detected at a wavelength of 222 nm. Retention times of Delamanid were found to be 4.541± 0.12 min, respectively. Total chromatographic runtime was 6 min.

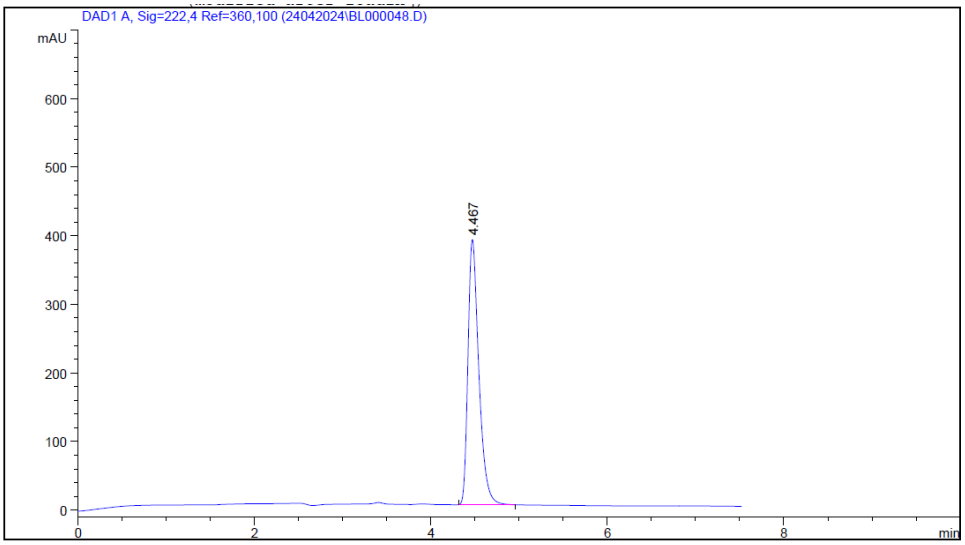

Figure 2: The chromatogram of a standard preparation

Parameter	Conditions
Type of system	Isocratic
Mobile phase	Methanol; 0.1% ortho phosphoric acid (81/19v/v)
Column	C18
Detection wavelength	252 nm
Flowrate	1.1 Ml/min
Volume of injection	20ul
Temperature of column	25
Pump mode	Isocratic
Run time	6
Retention time	4.541 ± 0.12 min

Method Validation

Specificity:

No interference from blank at retention time of delamanid peak, indicating that method is specific. Following figure show result of specificity:

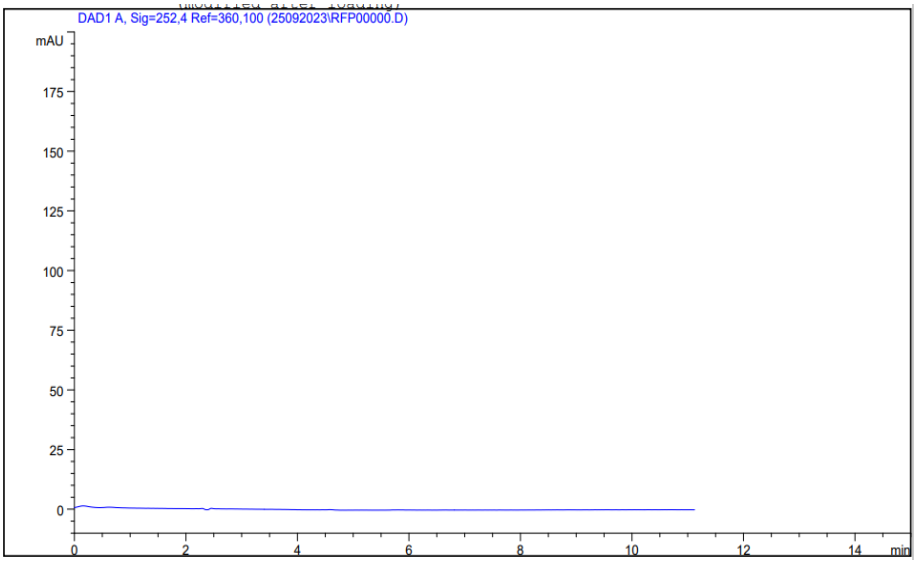

Figure 3: Chromatogram of Specificity

Linearity

The method is found to be linear in the concentration range of 10 – 50 µg/mL for Delamanid. The regression coefficient was found to be 0.999 (r2<1), Hence the method was linear within given range. Linearity results for Rifapentine are shown in Table.

Table 1: Linearity

Concentration µg/mL (n=5)	Peak area mAU*s (mean ± SD)
10	547.86 ±511.56
20	1051.03±1014.73
30	1587.20±1550.90
40	2118.90±2082.60
50	2582.62±2546.32

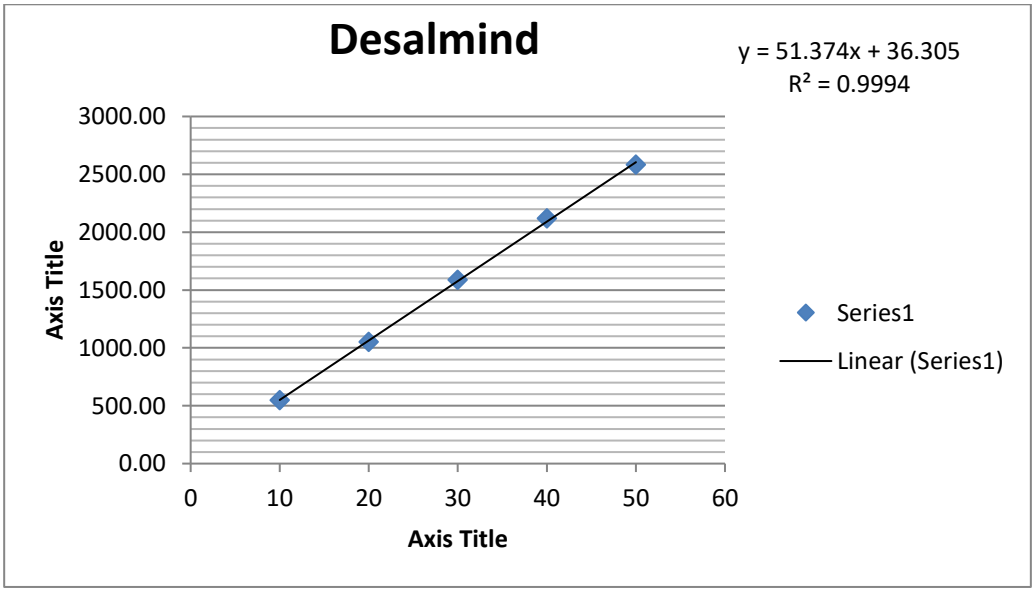

Figure 4: Linearity graph of Delamanid

Precision

As per ICH guidelines the limit for precision is NMT 2% RSD, the above developed method shows the precision of 0.29%RSD and 0.09%RSD which complies with the ICH guidelines. Hence the method was precise.

Table 2: Precision results

	Concentration µg/mL (n=3)	Peak area mAU*s (Mean ± SD)	%RSD
Interday Precision	20	1060.05±0.98	0.09
	30	1588.42±1.15	0.07
	40	2118.39±0.82	0.04
Interaday Precision	20	1063.31±3.09	0.29
	30	1585.72±1.63	0.1
	40	2109.51±1.46	0.07

Accuracy

The percentage recoveries of the results indicate that the recoveries are well within acceptance range (RSD<2) therefore method is accurate.

Table 3: Accuracy results

Sample No.	Spike level	Amount (µg/ml) Added	Amount (µg/ml) found	% Recovery	Mean Recovery	%	SD	%RSD
1.	80	18	18.0513	100.6406	100.1798		0.4219	0.4212
	80	18	17.9849	99.8123				
	80	18	18.0069	100.0866				
2.	100	20	20.0918	100.9185	100.7855		0.2275	0.2257
	100	20	20.0522	100.5229				
	100	20	20.09	100.9152				
3.	120	22	22.0516	100.4307	100.8556		0.3929	0.3895
			22.1446	101.2057				
			22.1116	100.9303				

LOD and LOQ

LOD was found to be 0.13 µg/ml, LOQ was found to be 0.38µg/ml. Hence; the developed method was validated for all the above parameters.

System suitability

Retention time, theoretical plate and tailing factor are within required limit.

Table 4: System suitability results

Property	Values	Required limits
Retention time	4.541	---------
Theoretical plates	5309	More than 2000

For the proposed RP-HPLC method, characteristic parameters were shown in Table:

Table 5: Characteristic parameters of Delamanid for the proposed RP-HPLC method

Parameters	RP-HPLC
Calibration range	10 to 50µg/ml.
Detection wavelength	222 nm

Mobile phase	Methanol; 0.1% ortho phosphoric acid (81:19v/v).
Retention time	4.541 ± 0.12 min
Regression equation	y = 51.374x + 36.305
Slope	46.24
Intercept	10.15
Correlation coefficient(r2)	0.999
Intraday precision (%RSD)	0.29
Interday precision(%RSD)	0.09
Limit of detection	0.13(µg/ml)
Limit of quantitation	0.38 (µg/ml)

Discussion

The developed RP-HPLC method for estimation of Delamanid was found to be reliable, accurate and precise. The method's specificity, linearity, accuracy, precision and robustness were all within acceptable limits. The method's simplicity, rapidity, and cost effectiveness make it suitable for routine analysis of Delamanid in pharmaceutical product.

Conclusion

For quantifying Delamanid in pharmaceutical formulations, the proposed method is easy, quick, precise, and sensitive. An easy mobile phase is used in this technique. Unlike several of the previously mentioned approaches, this one can be used without a buffer, needs no intricate sample preparation steps, and has a better sensitivity. In order to determine Delamanid, this proposed method can there for be used widely in quality control laboratories.

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