DEVELOPMENT AND VALIDATION OF STABILITY INDICATING ASSAY BY HPLC FOR ESTIMATION OF DELAMANID

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Abstract

Establishing quality and quantity is one of the prime concerns of any pharmaceutical industry for successful release of drug into the market. Not only for release of drug in to the market, had assurance on quality and quantity also stressed for safe human consumption of drugs. These two parameters i.e. quality and quantity are well confirmed in various types of bulk and commercial Pharma products by using different analytical techniques starting from oldest titrimetric analytical techniques to recently developed hyphenated techniques. A stability indicating analytical methods has been developed and validated for Delamanid as per International Conference on Harmonization. The Delamanid standard was exposed to acid and alkaline hydrolysis, oxidation, photolytic and thermal degradation conditionand separated using reversed phase BDS Hypersil C18 Column (250 × 4.6 mm, 5 µ; SN:10818991) , Mobile Phase: Acetonitrile: Water (80:20 v/v),Flow Rate: 1 ml/min, Column Temperature: Ambient , PDA Detection at 248 nm RT (min): 3.468 Asymmetry: 1.08 Plates (N): 3548. This method was validated for linearity, precision, accuracy, ruggedness and robusteness. Results obtained after validation study indicating that the proposed single method allowed analysis of Delamanid in the presence of their degradation products formed under a variety of stress conditions.

Keywords: Delamanid, HPLC, stability indicating methods, Forced degradation

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1. Introduction

Delamanid is a dihydro-nitroimidazooxazole derivative. Chemically it 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.

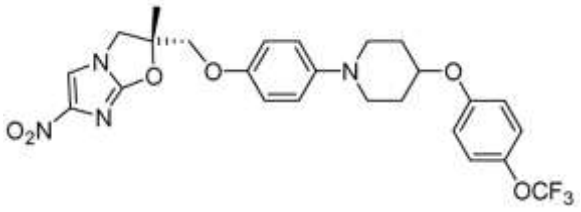


Figure 1: Structure of Delamanid

It acts by inhibiting the synthesis of mycobacterial cell wall components, methoxymycolic acid and ketomycolicacid. Delamanid is a pro-drug which gets activated by the enzyme deazaflavin dependent nitro reductase (Rv3547). It is widely used drug in the treatment of Multi drug resistant Tuberculosis (MDR TB).[1]

The drugs with few analytical methods were in a need of more simple and accurate analytical methods. The present research was attempted so as to develop and validate new stability indicating analytical methods. The principle intention of planning this endeavor is to deliver simple, accurate, precise, specific, reproducible,
robust, economical and highly sensitive methods for Delamanid. [2-3]

2. Material and Instrument

Reagents and chemicals: Methanol (AR Grade), Acetonitrile (HPLC Grade),NaOH (AR Grade) HCl (AR Grade) 30% H2O2 (AR Grade) HPLC grade water. All chemicals and reagents that is Methanol, Sodium hydroxide (NaOH), Hydrochloric acid (HCl), cetonitrile (ACN), Hydrogen peroxide solution 30% w/v (H2O2) were bought from LOBA CHEME PVT. LTD., Mumbai.

Instruments:
Analysis performed on HPLC instrument equipped with Borwin- PDA software (version 1.50) , Model PU 2080 Plus Intelligent HPLC pump, MX-2080-31 Solvent Mixing Module, Rheodyne sample injection port with 20µl loop, BDS Hypersil C18 Column (250 × 4.6 mm, 5 µ), MD 2010 Plus Multi-wavelength PDA detector.
UV-Visible Double beam spectrophotometer (Model JASCO V-730) , Shimadzu (model AY-120) Electronic weighting balance,
Sonicator: Prama solutions for laboratory, Elga Lab (PURELAB UHQ-II) water purification system. Conductivity below 0.05 µS/cm , Photo stability chamber- Newtronic Electronic pH meter, Calibrated Glasswares.

Preparation of Standard Stock Solution
An accurately weighed 10 mg of Delamanid was transferred to 10 ml volumetric flask, and the volume was made up to 10 ml with acetonitrile, to get standard stock solution of Delamanid(1000 µg/ml). From the standard stock solution,working standard solution was prepared using mobile phase as final diluent.
Selection of Analytical Wavelength: A solution of 20 µg/ml was prepared from standard stock solution of Delamanid (1000 µg/ml) and scanned over 200- 400 nm in UV– Spectrophotometer. The maximum absorbance was shown at 248 nm. Hence it was selected as analytical wavelength; UV spectrum is given in Fig.2

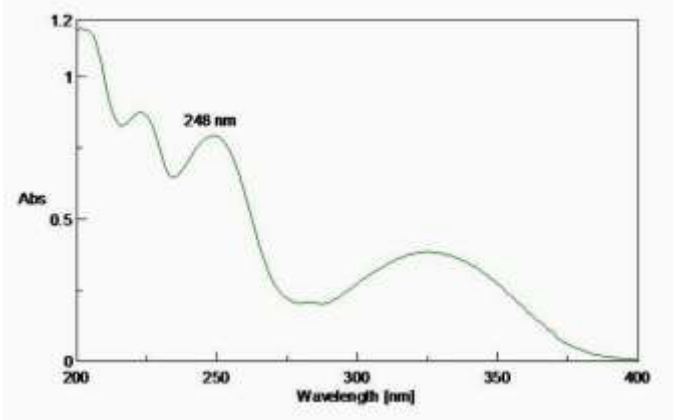
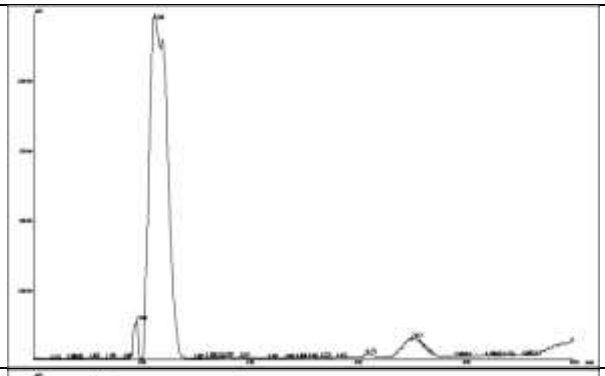
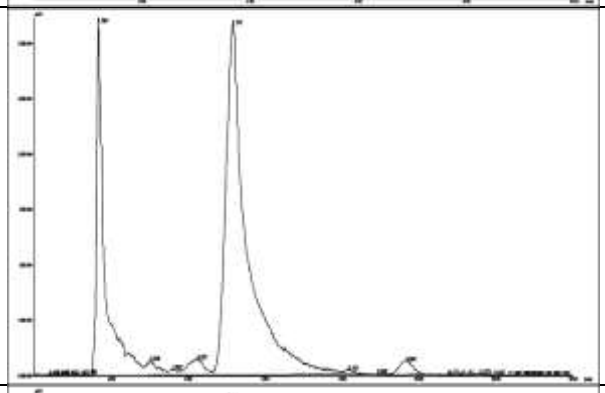
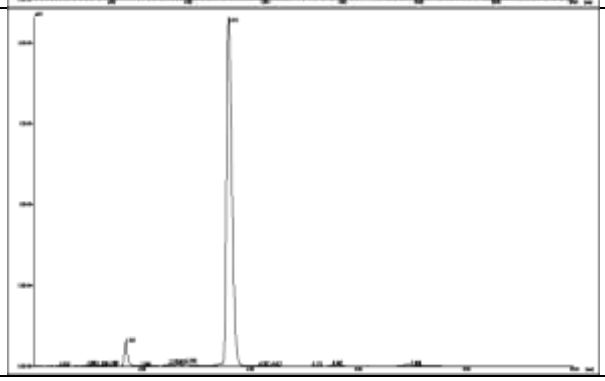


Fig 2: UV- Spectrum of Delamanid (20 µg/ml)

Mobile Phase Optimisation: To achieve optimum chromatographic condition various mobile phases were checked. ACN: Phosporic Acid (50: 50 v/v) system was initially tried but did not get a considerable number of theoretical plates. ACN has been chosen as organic modifier here. The water was subsequently tried with ACN (20:80 v/v) has obtained considerable theoretical plates and appropriate peak shape, with appropriate system suitability parameters.

Table 1: Trials of mobile phase for Delamanid

Sr. No.	Column and M.P.	Observation	Chromatogram(248 nm)
1.	ACN: Phosphoric acid (50:50 v/v)	Peak was not found.	
2.	ACN:Water (60:40 v/v)	Peak Tailing observed	
3.	ACN:Water (80:20 v/v)	R.T 3.4 min Good shape of peak	

Optimized Chromatographic Condition: Column: BDS Hypersil C18 Column (250 × 4.6 mm, 5 µ; SN:10818991) , Mobile Phase: Acetonitrile: Water (80:20 v/v),Flow Rate: 1 ml/min, Column Temperature: Ambient , PDA Detection at 248 nm RT (min): 3.468 Asymmetry: 1.08 Plates (N): 3548

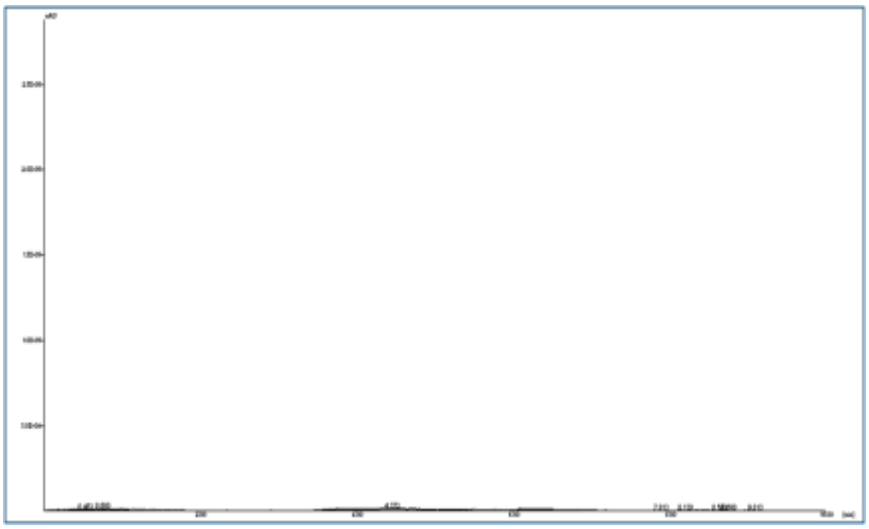


Fig. 2: Chromatogram mobile phase blank

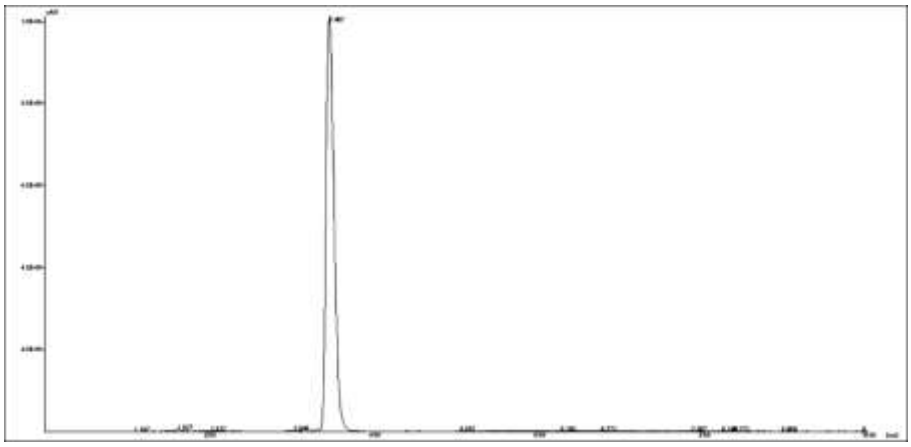


Fig. 3: Chromatogram of Delamanid

Validation of Analytical Method: [4-5]
The developed and optimized method was validated as per ICH Q2 (R1) guidelines. The method was validated in terms of specificity, system suitability, linearity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ) and robustness,

Specificity: Specificity was checked by injecting blank, placebo and comparing the peaks observed in sample solution with standard solution. No interference was observed. Observed peak in sample solution matched standard peak of Delamanid showed that, the method is specific.

System suitability: System suitability performance was evaluated by system suitability parameters such as retention time, theoretical plates, asymmetric factor and the method indicated good performance of the system as depicted in Table.2

Table 2: System suitability parameters

Concentration µg/ml	**RT (min)**	**Area**	**Plates**	**Asymmetry**
10	6.587 ± 0.528	939125.23	6874.22	1.11

Linearity
Linearity is the ability of the analytical method to obtain results that are directly proportional to concentration of the analyte in the sample.

From the standard stock solution (1000 µg/ml) of Delamanid, solution was prepared containing 100 µg/ml in Acetonitrile. This solution was further used to prepare range of solution containing six different concentrations. The linearity (relationship between peak area and concentration) was determined by analyzing six solutions over the concentration range of 5-30 µg/ml, the equation of calibration curve was found to be y = 85096 x + 17796.

The results obtained are shown in Table 3 for Delamanid. The peak area of drug was plotted against the corresponding concentrations to obtain the calibration curve as shown in Fig. 4 .

Table 3: Linearity study of Delamanid

Conc (µg/ ml)	L1	L2	L3	L4	L5	L6	Avg AREA	SD	% RSD
5	385485.26	382593.25	379590.5	387547.25	387013.3	384100.5	384388.343	2979.826	0.775
10	939125.23	957953.982	965685.684	962176.5	962874.8	964915.5	958788.616	10006.281	1.044
15	1266604.056	1294668.81	1309146.99	1264362.75	1299816.5	1289500.5	1287349.934	18152.245	1.410
20	1738074.647	1681236.7	1722604.67	1700636	1696519.7	1683055.25	1703687.827	22497.788	1.321
25	2115450	2162710.47	2161572.36	2142322.5	2114020.5	2097242.52	2132219.685	27309.986	1.281
30	2555594	2607634.17	2597655.5	2555706.75	2578199.9	2557738	2575421.385	22953.140	0.891

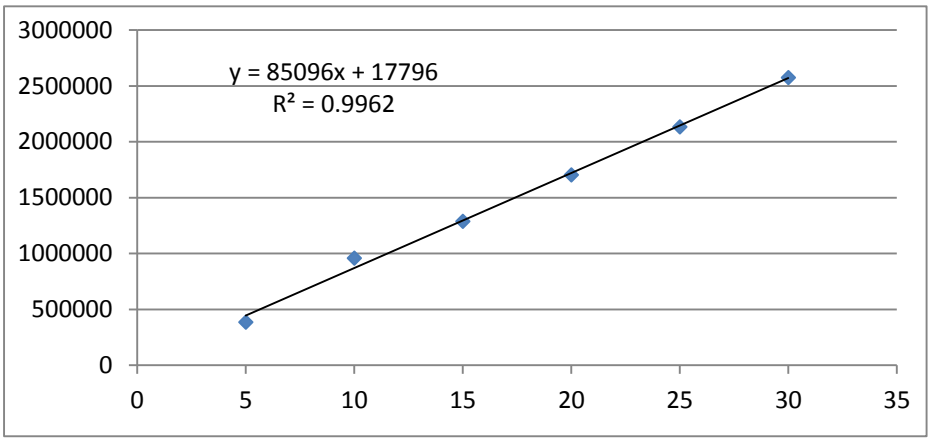


Fig 4 : Linearity curve of Delamanid (5-30 µg/ml)

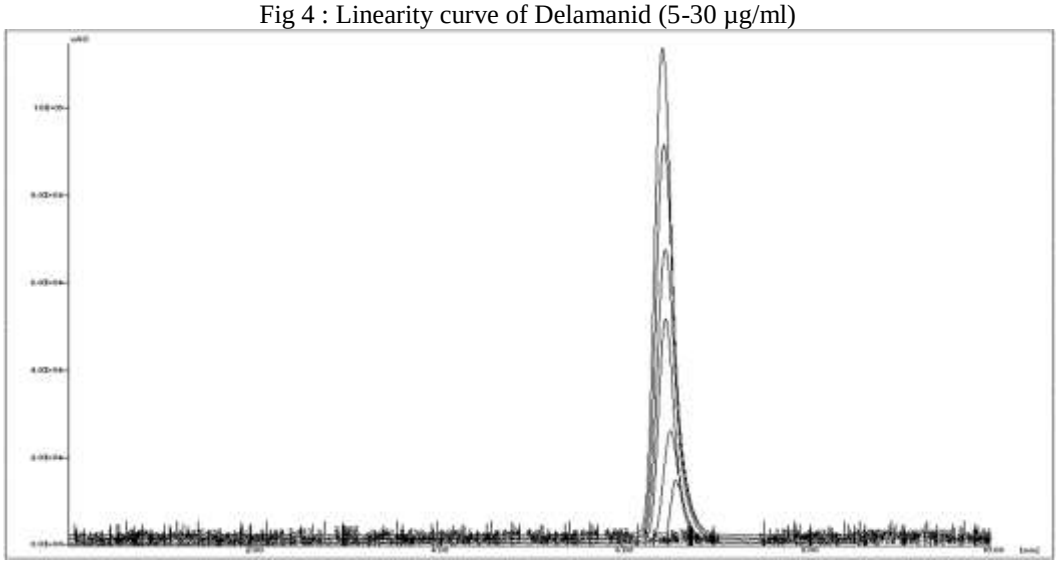


Fig 5: Chromatogram of Overlay Spectra of linearity of Delamanid

Precision

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the Intra-day studies, 3 replicates of 3 different concentrations were analyzed in a day and percentage RSD was calculated. For the inter day variation studies, 3 different concentrations were

analyzed on 3 consecutive days and percentage RSD was calculated. The results obtained for intraday and inter day variations are shown in Table 4 and 5 , respectively

Table 4 : Intra-day precision study of Delamanid

SN	Theroticalconc	Area	Practical Conc	% x	AVG	SD	%RSD
1	10	873872.399	10.060	100.601			
2	10	870489.62	10.020	100.204	100.446	0.212	0.211
3	10	873286.214	10.053	100.532			
4	15	1306635.33	15.146	100.971			
5	15	1301350.05	15.084	100.557	100.571	0.394	0.392
6	15	1296586.291	15.028	100.184			
7	20	1728784.057	20.107	100.533			
8	20	1736383.649	20.196	100.979	100.687	0.254	0.252
9	20	1729043.316	20.110	100.548			

Table 5: Inter-day precision of Delamanid

SN	Theroticalconc	Area	Practical Conc	% x	AVG	SD	%RSD
1	10	865685.684	9.964	99.639			
2	10	872176.500	10.040	100.402	100.255	0.557	0.556
3	10	874915.500	10.072	100.724			
4	15	1309146.991	15.175	101.168			
5	15	1284362.750	14.884	99.227	100.008	1.025	1.025
6	15	1289500.500	14.944	99.629			
7	20	1722604.667	20.034	100.170			
8	20	1700636.000	19.776	98.879	99.552	0.647	0.650
9	20	1713055.250	19.922	99.609			

Limit of detection (LOD) and limit of quantitation (LOQ)
LOD and LOQ are calculated from the formula: -

$$LOD = \frac{3.3\ \sigma}{S} \qquad LOQ = \frac{10\ \sigma}{S}$$

Where,
σ = S.D of the response at lowest concentration or standard deviation of Y intercept;
S = Average of slope of the calibration curve
LOD of Delamanid = 0.390 µg/ml
LOQ of Delamanid = 1.181 µg/ml
Specificity : The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 998, indicating the no interference of any other peak of degradation product, impurity or matrix. (Table 6).

Table 6 : Peak purity of Delamanid HCl

Drug	Purity tail	Purity front
Delamanid	998.56	998.38

Assay
Tablets with label claim 50 mg of Delamanid (DELTYBA Tablets 50 mg) were weighed and powdered. A quantity of powder equivalent to 10 mg of Delamanid was transferred to a 10 ml volumetric flask containing 5 ml of Acetonitrile. The mixture was ultra-sonicated for 10 min and the resulting sample stock solution was filtered with

Whatman filter paper 41 and the volume was made up with the Acetonitrile to get concentration of 1000 µg/ml. Further dilutions were made to get concentration 10 µg/ml which was injected on system. Procedure was repeated for six times. Sample solution was injected and area was recorded. Concentration and % recovery was determined from linear equation. The results obtained are shown in Table 7.

Table 7: Assay of marketed formulation

Sr. No.	**Peak Area**	**Amount Recovered (µg/ml)**	**%Recovery**	**Mean ± % RSD**
1	868094.750	9.992	99.922	100.465 ± 0.817
2	864060.750	9.945	99.448	
3	878832.250	10.118	101.184	
4	869656.433	10.011	100.106	
5	872891.500	10.049	100.486	
6	882749.000	10.164	101.644	

Accuracy

To check accuracy of the method, recovery studies were carried by spiking the standard drug to the tablet sample solution, at three different levels around 50, 100 and 150 %.Basic concentration of sample solution chosen was 10 µg/ml. % recovery was determined from linearity equation. The results obtained are shown in Table 8.

Table 8: Accuracy of Delamanid

Level	**Conc. of Sample solution (µg/ml)**	**Conc. of Standard solution spiked (µg/ml)**	**Area**	**Amount recovered (µg/ml)**	**% Recovery**	**% Recovery (Mean ± %RSD)**
50 %	10	5	1294633.250	15.005	100.031	100.825 ± 0.964
			1318601.000	15.286	101.909	
			1301048.250	15.080	100.534	
100 %	10	10	1725508.500	20.068	100.340	99.628 ± 0.639
			1704652.253	19.823	99.115	
			1710013.327	19.886	99.430	
150 %	10	15	2147504.750	25.027	100.109	100.395 ± 1.173
			2181133.500	25.422	101.689	
			2132175.250	24.847	99.388	

Robustness

Robustness of the method was checked by carrying out the analysis under conditions during which mobile phase composition (± 2 ml Composition), detection wavelength (± 1 nm), flow rate (± 0.05 ml/min) were changed and the effect on the area were noted. Robustness of the method checked after deliberate alterations of the analyticalparameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters indicating that the method is robust.

Table 9: Robustness study

% RSD Found for Robustness Study (Peak Area)		
MP Composition (± 2 ml Composition)	**Detection Wavelength (± 1 nm)**	**Flow Rate (± 0.05 ml/min)**

82:18	80:20	78:22	247	248	249	0.95	1	1.05
0.742	0.951	0.229	0.954	0.625	1.467	0.690	0.779	0.422

3. RESULT AND DISCUSSION

Table 10: Summary of Validation Parameters by HPLC

Sr. No.	Validation parameters	Delamanid Results
1.	Linearity equation R^2 Range	y = 85096 x + 17796 R^2= 0.9962 5-30 µg/ml
2.	Precision	(%RSD)
	Intraday	0.211 – 0.392
	Interday	0.556 – 1.025
3.	Assay	100.465 ± 0.817
4.	Accuracy	Mean ± %RSD
	50	100.825 ± 0.964
	100	99.628 ± 0.639
	150	100.395 ± 1.173
5.	Limit of detection	0.808 µg/ml
6.	Limit of quantitation	2.450 µg/ml
7.	Specificity	Specific
8.	Robustness	Robust

Forced Degradation Studies

The effect of different environmental factors on drug stability and quality must be checked. Thus, the drug was kept to various stress conditions for varying periods of time, using various strengths of reagents. Conditions were tried to optimize to achieve recovery of 70- 90%. The Delamanid standard was exposed to acid and alkaline hydrolysis, oxidation, photolytic and thermal degradation condition. All studies were done at 100 µg/ml concentration of sample. Summary of forced degradation shown in Table 11.

Acid Catalyzed Hydrolysis Degradation

Sample was made by adding 1 ml of 1 N HCl to 1 ml stock solution (1000 µg/ml) of Delamanid. Solution was placed at room temperature for about 1 hour. Solution was then neutralized and volume made to 10 ml with mobile phase and injected to system. Chromatogram was shown in Fig. 6.

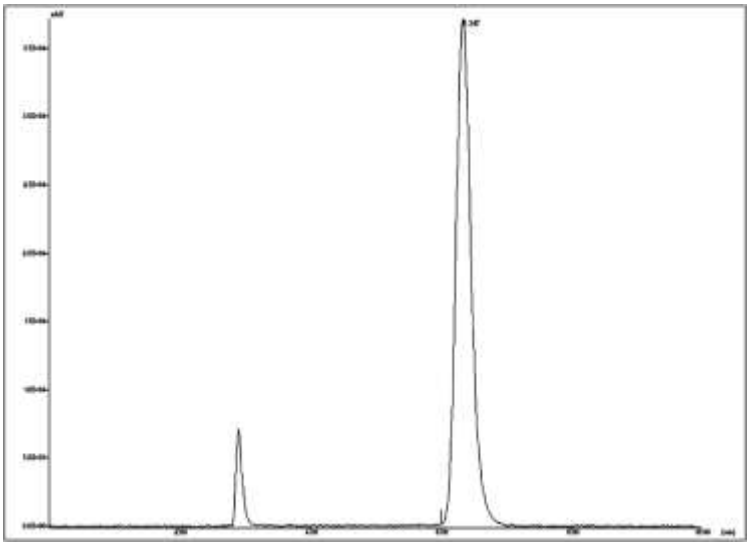


Fig. 6: Acid hydrolysis Chromatogram of Delamanid

Alkali Catalyzed Hydrolysis Degradation:

Sample was made by adding 1 ml of 1 N NaOH to 1 ml stock solution (1000 µg/ml) of Delamanid. Solution was placed at room temperature for about 1 hour. Solution was then neutralized and volume made to 10 ml with mobile phase and injected to system. Chromatogram was shown in Fig. 7.

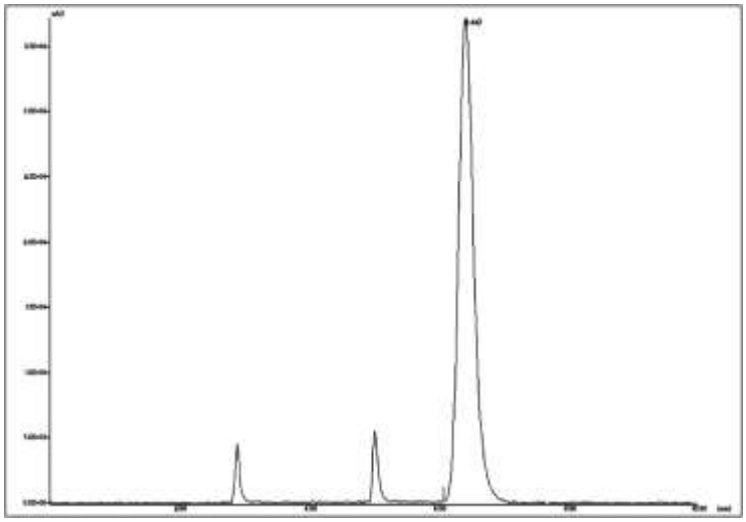


Fig. 7:Alkali hydrolysis Chromatogram of Delamanid

- **Hydrogen-Peroxide Induced Degradation:**

Sample was made by adding 1 ml of 30 % w/v H_2O_2 to 1 ml stock solution (1000 µg/ml) of Delamanid. Solution was placed at room temperature for about 1 hour. Volume was then made to 10 ml with mobile phase and injected to system. Chromatogram was shown in Fig. 8.

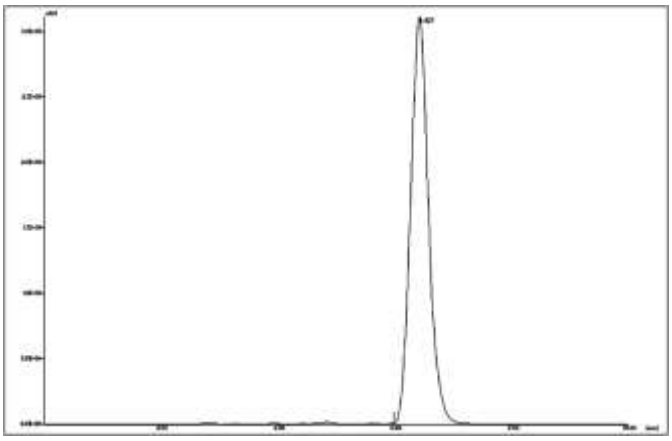


Fig. 8: Thermal degradation Chromatogram of Delamanid

Photolytic Degradation:

Sample was exposed to UV light for not less than 200-watt hours/square meter followed by white fluorescent light of illumination for not less than 1.2 million lux hours. After exposure 10 mg of powder was weighed and dissolved in acetonitrile to 10 ml. From this final dilution of concentration 100µg/ml was prepared and injected to get chromatogram. Chromatogram was shown in Fig. 9.

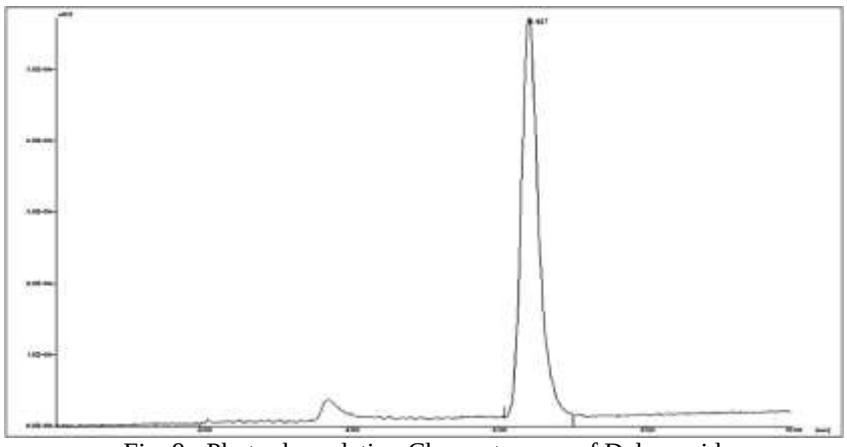


Fig. 9: Photo degradation Chromatogram of Delamanid

Table 11: Summary of stressed degradation

SN	Parameter and Condition	% Recovery	% Degradation	RT of degraded products
1	Acid hydrolysis (1 N HCl for 1 Hr.)	81.67	18.33	DP1 – 2.916 min
2	Alkaline Hydrolysis (1 N NaOH for 1 Hr.)	76.28	23.72	DP1- 2.912 min DP2 – 5.136 min
3	Oxidative Degradation (30 % w/v H_2O_2 for1Hr.)	97.64	2.36	--
4	Thermal degradation (100˚C for 4 Hr.)	98.89	1.11	--
5	Photo degradation (UV light200 Watt hours/square meter followed by fluorescence light of NLT 1.2 million Lux-Hr)	89.52	10.48	DP3 – 3.854 min

3. Conclusion

The developed HPLC technique is fast, simple, precise, specific, accurate, and stability-indicating. Validation of the method proved that the method is suitable for the analysis of Delamanid. The method is robust enough to reproduce accurate and precise results under different chromatographic conditions. Degradation studies confirmed the homogeneity and free of interferences with the peak of interest. The developed method can be used for routine analysis of Delamanid.

4. References

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