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Original Article

Stability indicating HPTLC method development and validation for the analysis of novel nitroimidazole antitubercular drug delamanid

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ABSTRACT

Background and Aims: Delamanid is an active drug substance used in efficient treatment for multidrug-resistant tuberculosis (MDR TB). A straightforward, accurate, and precise HPTLC technique has been developed and validated for the study of the delamanid.

Methods: The samples were placed as bands on an aluminium TLC plate covered with silica gel. Delamanid was completely separated using ethyl acetate and n-hexane as the mobile phase; the RF value was 0.51 0.098. At 330 nm, densitometric detection was performed in the absorbance mode. This method led to the discovery of sharp, symmetrical, and well-defined peaks.

Results: A linear correlation was obtained for the concentration range of 200–1200 ng/spot, with a determination coefficient of 0.992. According to the requirements set out by the International Conference on Harmonisation, the method's accuracy, recovery, repeatability, and robustness were all validated. The limit of quantitation was determined to be 349.11 ng/spot, whereas the lowest detectable level was found to be 115.2 ng/spot. This approach permitted the analysis of delamanid in the presence of their degradation products created under various stress conditions, according to the findings of the validation research. delamanid degraded by 20.54% and 35.72% under alkaline and photodegradation conditions, respectively.

Conclusion: The established method might be used to evaluate the stability of delamanid in a commercial pharmaceutical dose form. Regarding HPTLC-induced degradation of delamanid, no prior technique has been documented. This technique was successfully used to quantify the amount of delamanid in its commercially available formulation.

Keywords: HPTLC, Method Development, Validation, Stability indicating method, Delamanid

INTRODUCTION

Delamanid is an effective substance used in the treatment of MDR TB. ®Deltyba is trade name of it. It is the foremost in a new class of TB drugs assigned as nitroimidazole. Chemically, Delamanid is (2R)-2-Methyl-6- nitro-2-[(4-{4-[4-(trifluoromethoxy)phenoxy]-1- piperidinyl} phenoxy) methyl]-2,3-dihydroimidazo[2,1- b][1,3]oxazole, as shown in Figure 1. Delamanid is used to treat people with pulmonary tuberculosis (TB) that is multidrug resistant. By preventing the production of mycobacterial cell wall constituents such as methoxymycolic acid and meromycolic acid, it functions as a prodrug (Field, 2013). For its analysis, no HPTLC technique is available. The objective of this study was to create a repeatable, exact, accurate, and specific HPTLC technique for investigating delamanid. The. According to the ICH guidelines, the developed technique was validated using the criteria of linearity, accuracy, precision, robustness, ruggedness, LOD, and LOQ (Field, 2013; Bahuguna & Rawat, 2020)

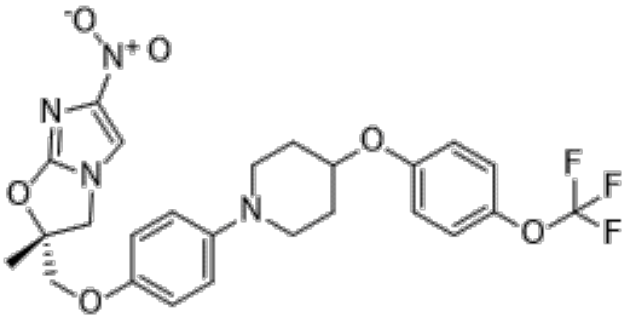

Figure 1. Structure of the Delamanid

Thin layer chromatography is a form of chromatography that

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separates and analyzes mixtures using an inert backing and a thin stationary phase. Since the mid-1970s, with the introduction of contemporary methodological and instrumental advancements in TLC (known as high performance TLC), the use of this method in qualitative and quantitative analysis of pharmaceutical, environmental, toxicological, food, and agricultural samples has steadily increased. This tendency persists if certain quality control laboratories now frequently use quantitative HPTLC because of this pattern. The following are the main benefits of HPTLC: ease of use, minimal pre-treatment, effectiveness with small amounts of sample, parallel analysis of samples (up to 72 samples can be analysed concurrently and under identical conditions on a 20cm plate), numerous nondestructive detection methods (such as visualization and scan in visible or UV light at different wavelengths), a wide range of developing solvent options and low consumption of solvents. (In fact, the expenses associated with providing solvents and maintenance are much lower compared with HPLC). These benefits, with the method's dependability, sensitivity, and repeatability, make it a viable option for other chromatographic processes like HPLC. In the current work, a straightforward and practical analytical procedure using the HPTLC-densitometric method is described for the identification and quantitative measurement of delamanid in bulk drugs (Le Roux, Wium, Joubert, & Van Jaarsveld, 1992).

MATERIALS AND METHODS

Delamanid pure drug was obtained as a gift sample from Mylan, Hyderabad. HPLC grade Ethyl acetate, methanol, and n-hexane were purchased from Qualigen (India) Ltd., Mumbai, India. All other chemicals are of analytical grade from S.D. Fine Chemical Ltd., World, India. and the volumetric glassware of class A grade were used throughout the experimental work.

Instrumentation

For applying the samples to the HPTLC plate, a Camag Linomat automated sample applicator with a Linomat 100-L syringe 695.0014 was used. Chromatographic separations were performed on 20 20 cm aluminium packed plates that had silica layers pre-coated with 0.2 mm. The plates were developed ascendingly in a Camag twin-trough chamber with a stainless steel top for 20 20 cm plates. On a Camag TLC scanner III running WINCAT software with a D2lamp as the radiation source, densitometric scanning was performed (Ferenczi-Fodor, Renger, & Végh, 2010).

HPTLC analysis

Preconditioning: After selection of the chromatographic layer, plates were prewashed with methanol and then activated at 70°C for 60 min.

Sample application

The samples were spotted using an automated applicator at a constant application rate of 5 s/µL in bands that were 6 mm wide. There were 9 mm between each ring. The distances from the plate's bottom and left edge were maintained at 30 and 20 mm, respectively. Samples were placed under a continuous nitrogen gas drying stream at a constant 150 nL/s application rate.

Selection of suitable mobile phase

Different solvents like acetonitrile, petroleum a ether, formic acid, and methanol were tried in various combinations and compositions. Among them, ethyl acetate: n-hexane (70:30) solvent mixtures was selected for the method development.

Chromatographic development

The tank was saturated for 20 min before the spotted plate was inserted. Under tight light-protected conditions, 10 mL of the mobile phase was used to develop plates. Around 80 mm of development distance was present. Chromatography was conducted at a temperature of 25°C and a relative humidity of 33%.

Detection and scanning

After development, the plate was dried with a dryer for 1 min. Densitometric scanning was then performed in the absorbance mode using a D2 light source at 330 nm (λmax for the compounds). The monochromator bandwidth was maintained at 6 mm, and the dimension of the slit was set at 0.30 mm.

Preparation of stock and working solutions

A stock solution was prepared (1000 µg/mL) by dissolving and diluting 10 mg of delamanid in 10 mL methanol. The solution was sonicated for 10 min.

Calibration curve

The concentration 0.2 µL, 0.4 µL, 0.6 µL, 0.8 µL, 1 µL, 1.2 µL were applied in three replicates on the TLC plate. The spotted plates were developed and scanned as described above. The calibration curve was constructed by plotting average peak areas versus the corresponding amounts, and the regression equation was calculated for Delamanid.

RESULTS AND DISCUSSION

Method Development and Optimisation

Various chromatographic conditions were investigated to attain satisfactory results for the Delamanid qualitative and quantitative analyses. Developing the mobile phase individually on glass and aluminium TLC plate and comparing the results indicated that using aluminium backing plates produces well-defined spots with better resolution. Hence, in this work, aluminium sheet plates recoated with silica gel were selected as the stationary phase.

Method validation

HPTLC was performed as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) linearity, accuracy, precision, robustness, specificity, limit of detection (LOD), and limit of quantitation (LOQ) (ICH, 2005).

Linearity

The linearity of the method was evaluated by constructing calibration curves at six concentration levels. The calibration curve (Figure 2) was plotted over a concentration range of 200–1200 ng/spot (Table 1). Aliquots of the standard working solution of delamanid were applied to the plate (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 µL/spot). The calibration curves were developed by plotting peak area versus concentrations (n = 6) with the help of the win CATS software. We obtained the linear equation y = 2.6001x + 165.72, where y is area and x is concentration. Figures 3 and 4 show the 2D Densitogram of the Delamanid and the 3D Densitogram of the Delamanid Standard (Rf 0.51 ± 0.098) respectively.

Table 1. Calibration parameters

Sr. No.	Conc.(ng/spot)	Area
1	200	602.4
2	400	1210.1
3	600	1771.5
4	800	2351.4
5	1000	2816.9
6	1200	3162.5

Precision

To assess the three sources of variance, accuracy was measured at three distinct concentration levels of 0.6, 0.8, and 1 µL/spot. When a densitometer measures the same location three times, the accuracy was initially evaluated. The same analyst on two separate days assessed repeatability under the same conditions.

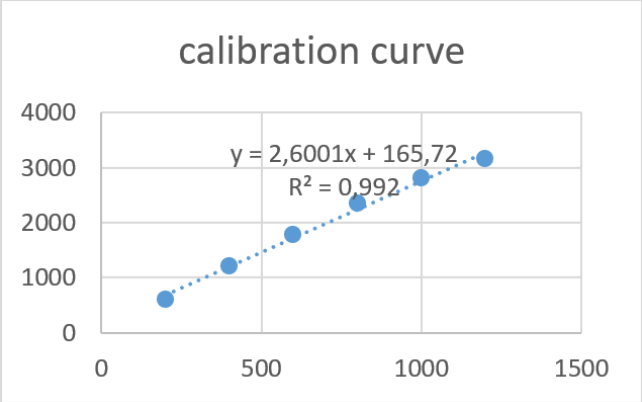

Figure 2. Calibration curve for Delamaid

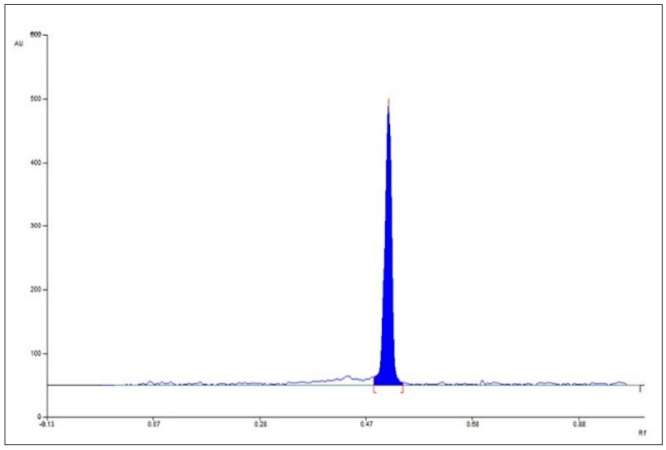

Figure 3. 2D Densitogram of Delamanid

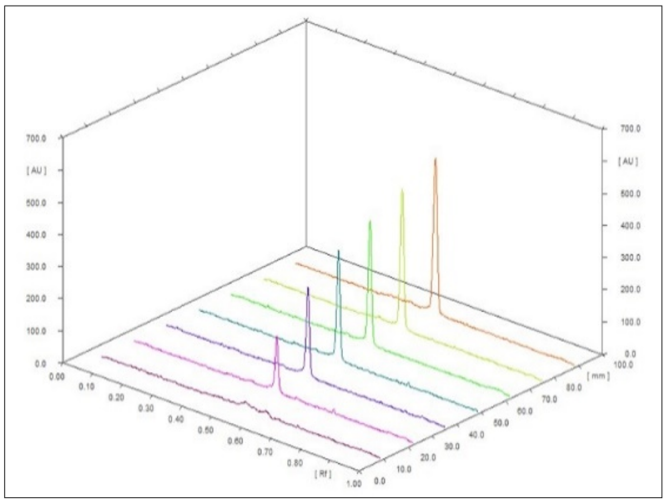

Figure 4. 3D Densitogram of Delamanid Standard (Rf 0.51 ± 0.098)

Second, the same answer is found thrice. The RSD values were under 3%, confirming both the appropriate intermediate accu-

racy and the strong repeatability of sample application and peak area measurement. RSD values between 1% and 5% in TLC densitometry are considered acceptable (Tables 2 and 3).

Accuracy (Recovery studies)

Recovery studies were carried out to assess the accuracy of the method. These studies were conducted at three levels. The percentage recovery was found to be within the limits shown in Table 4.

Robustness

Robustness was determined by altering chromatographic conditions like mobile phase composition as ethyl acetate: n-hexane (60:40). The low value of RSD indicates robustness of the method. The results are shown in Table 5.

LOD and LOQ

The sensitivity of the proposed method was estimated in terms of the limit of detection (LOD) and limit of quantitation. The ICH indicates that LOD (which they call 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. 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 analyte.(Table 6)

Stress degradation studies of bulk drug

Stability studies were conducted to provide evidence on how the quality of drug varies under the influence of a variety of environmental conditions like hydrolysis, oxidation, and temperature. Dry heat and photolytic degradation were performed in the solid state.

Acid hydrolysis

To 1 ml standard stock solution of drug (1000 µg/ml), 1 ml of 0.1 N HCl was added and the volume was made to 10 ml with Acetonitrile to get 100 µg/ml solution. The solution was kept for 24 h in dark, neutralised with 0.1 N NaOH and 10 µl of this solution was applied to a TLC plate (1000 ng/band concentration). The acid degradation blank is prepared in the same way without using an analyte. Under acid hydrolysis, the percent recovery obtained for Delamanid was 98.74 % with no peak of degradants. A representative chromatogram is shown in Figure 5.

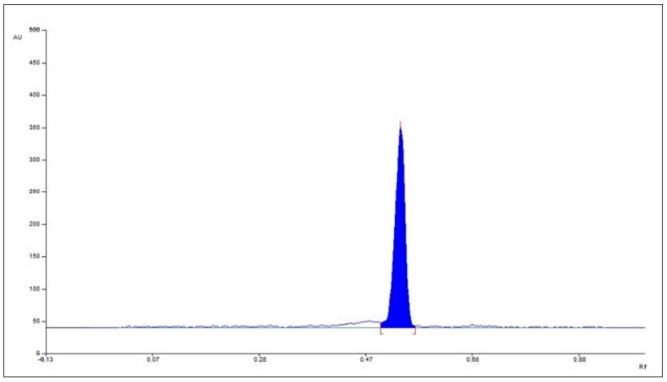

Figure 5. Densitogram of Delamanid after acid degradation

Alkaline hydrolysis

To 1 ml standard stock solution of drug (1000 µg/ml), 1 ml of 0.1 N NaOH was added, and the volume was made to 10 ml with Acetonitrile to get 100 µg/ml solution. The solution was kept for 24 h in dark, neutralised with 0.1 N HCl and 10 µl of this solution was applied to the TLC plate (1000 ng/band concentration). The alkali degradation blank is prepared in the same way without using an analyte. Under alkaline hydrolysis, the percent recovery obtained for delsmani was 79.46 % i.e.20.54 % degradation with one peak of degradants. A representative chromatogram is shown in Figure 6.

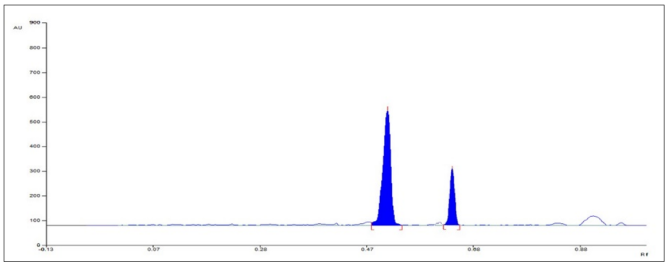

Figure 6. Densitogram of the Delamanid after alkaline degradation

Degradation under oxidative conditions

To 1 ml standard stock solution of drug (1000 µg/ml), 1 ml of 30 % H_2O_2 was added and the volume was made to 10 ml with Acetonitrile to get 100 µg/ml solution. The solution was kept for 24 h in dark and 10 µl of this solution was applied to the TLC plate (1000 ng/band concentration). The blank is prepared in the same way without using an analyte. Under oxidative degradation, the percent recovery obtained for Delamanid was 96.18% with no peak of degradants. A representative chromatogram is shown in Figure 7.

Table 2. Intraday precision results

Sr. no.	Conc. (ng/spot)	Area	Mean	S.D.	%RSD
1	600	1871.5	1872.53	17.6	0.9
2	600	1851.4			
3	600	1894.7			
4	800	2337.5	2348.16	7.73	0.32
5	800	2351.4			
6	800	2355.6			
7	1000	2775.6	2796.73	16.8	0.6
8	1000	2797.7			
9.	1000	2816.7			

Table 3. Interday precision results

Sr. no.	Conc.(ng/spot)	Area	Mean	S.D.	%RSD
1	600	1702.1	1724.1	21.0	1.2
2	600	1752.5			
3	600	1717.7			
4	800	2232.7	2258.6	29.0	1.3
5	800	2244.0			
6	800	2299.1			
7	1000	2655.8	2663.9	17.7	0.6
8	1000	2647.4			
9.	1000	2688.5			

Table 4. Results of recovery studies

%Recovery	Standard API	Admixture	Area	Mean	%Recovery.
50	0.4µl(400ng)	0.2 µl(200 ng)	1702.2	1728.36	100.1
50	0.4µl(400ng)	0.2 µl(200 ng)	1747.1		
50	0.4µl(400ng)	0.2 µl(200 ng)	1735.8		
100	0.4µl(400ng)	0.4 µl(200 ng)	2339.4	2318.83	103.5
100	0.4µl(400ng)	0.4 µl(200 ng)	2309.8		
100	0.4µl(400ng)	0.4 µl(200 ng)	2307.3		
150	0.4µl(400ng)	0.6 µl(200 ng)	2954.4	2922.03	106
150	0.4µl(400ng)	0.6 µl(200 ng)	2918.9		
150	0.4µl(400ng)	0.6 µl(200 ng)	2892.8		

Table 5. Robustness results

Conc.(µL)	Area	Mean	S.D.	R.S.D.
0.6 µL	1630.6	6329	18.3	1.1
0.6 µL	1592.6			
0.6 µL	1632.5			
0.8 µL	2424.6	8188.3	36.02	1.4
0.8 µL	2498.5			
0.8 µL	2419.8			
1.0 µL	2570.3	2560.96	18.7	0.7
1.0 µL	2534.8			
1.0 µL	2577.8			

Table 6. LOD and LOQ

Drug	LOD	LOQ
Delamanid	115.2 ng	349.11 ng

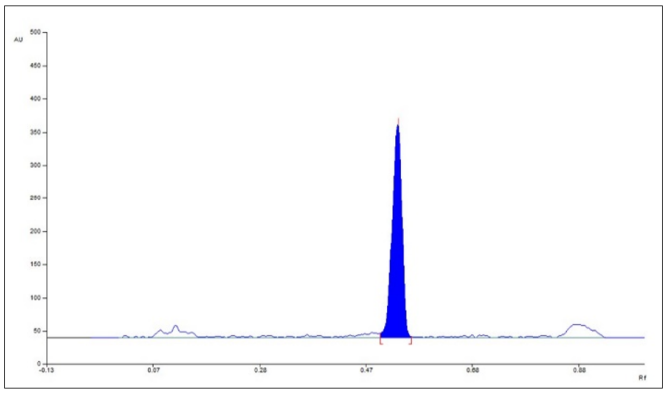

Figure 7. Densitogram of Delamanid after oxidation with 30 % v/v H_2O_2

Degradation under dry heat

In dry heat study drug sample is kept in an oven (1000 C) for a period of 2 h. A sample was withdrawn, 10 mg of it was dissolved in acetonitrile to obtain a solution of 1000 µg/ml and further diluted with Acetonitrile to get 100 µg/ml as final concentration and 10 µl of this solution was applied on a TLC plate (1000 ng/band concentration). Under dry heat degradation conditions, the percent recovery obtained for Delamanid was 98.93 % with no peak of degradants. A representative chromatogram is shown in Figure 8.

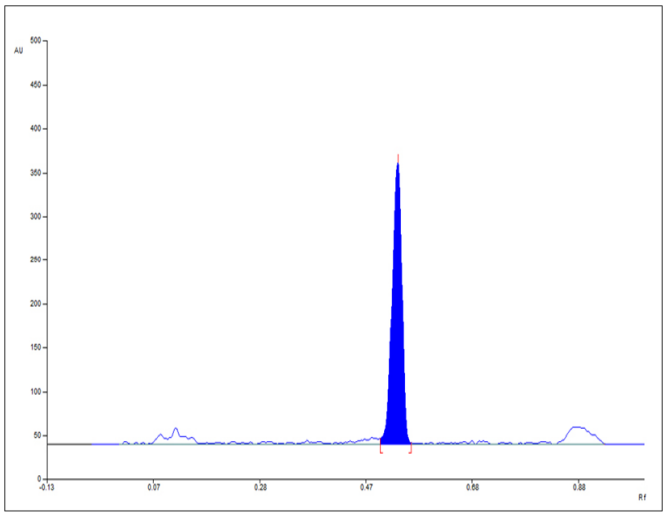

Figure 8. Densitogram of the Delamanid after dry heat degradation

Photo-degradation studies

The photodegradation stability of the drug was studied by exposing the drug to UV light providing illumination of NLT 200 watt hr/m^2 and exposure to cool white fluorescence light of NLT 1.2 million Lux-Hr. After exposure, 10 mg of drug was accurately weighed and transferred to 10 ml of volumetric flask; the volume was made up with Acetonitrile. Further dilution made with Acetonitrile to get 100 µg/ml as final concentration and 10 µl of this solution was applied to the TLC plate (1000 ng/band concentration). An average of 64.28 % of Delamanid was recovered i.e.35.72 % degraded with one peak of degradants after exposure to UV light, and an average of 99.08 % of Delamanid was recovered with no peak of degradants after exposure to fluorescence light. A representative chromatogram is shown in Figures 9 and 10, respectively (Guideline, 1996).

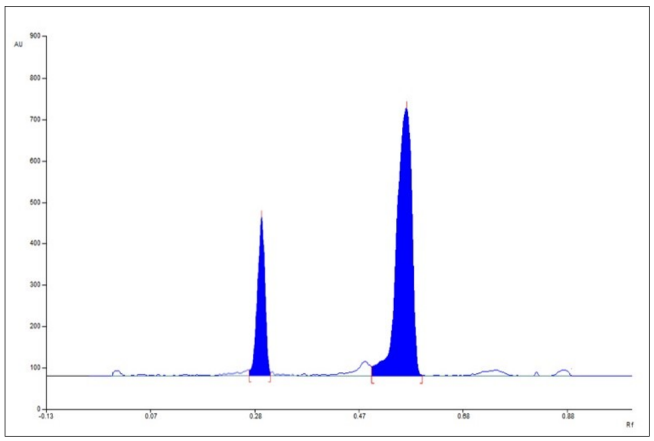

Figure 9. Densitogram of the Delamanid after UV illumination exposure

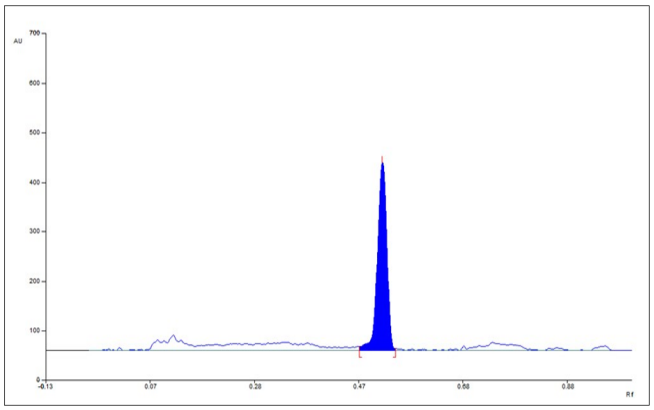

Figure 10. Densitogram of the Delamanid after fluorescent light exposure

Table 7. Summary of Degradation

Stress condition/Duration	% Recovery of the Analyte	RT of the degraded products
Acidic/2 N HClfor 24 h	98.74 %	-
Alkaline/1 N NaOH for 24 h	79.46 %	Degradant at Rf 0.65
Oxidative 30 % H_2O_2 at room temperature for 24 h	96.18 %	-
Dry heat / 100ºC/ 24 h	98.93 %	-
UV illumination NLT 200 W/m2	64.28 %	Degradant at Rf 0.29
Fluroscent light NLT 1.2 10^6 Lux h	99.08 %	-

CONCLUSION

For the examination of Delsmani, a new HPTLC densitometric technique was created, verified in accordance with the ICH criteria, and shown to be repeatable, precise, accurate, and robust. In comparison with other analytical techniques, the process is quick, easy, and moderately affordable. It is also practical enough to be recommended for the regular analysis of the delamanid. For the safety of patients, the quality of pharmaceutical goods is crucial. The presence of degradation chemicals or contaminants affects effectiveness and safety of medications. This study examined Delamanid's forced degradation under ICH-required conditions to examine its degradation profile and clarify the structures of the degradation products. A stability-indicating HPTLC technique that is green, sensitive, accurate, exact, economical, less time-consuming, cost-effective, and repeatable was created. The approaches were shown to be linear, exact, accurate, specific, and selective to the drug in the presence of degradation products in the validation trials. At room temperature, the medication is vulnerable to alkali hydrolytic and photolytic deterioration. The stability indication research was conducted in accordance with ICH Q1A (R2) recommendations.

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